STANDARDS FOR

FLUORIDE DENTIFRICES

THE PROPRIETARY ASSOCIATION SUBGROUP ON FLUORIDE DENTIFRICES

MARCH 11, 1978

THE PROPRIETARY ASSOCIATION

1700 Pennsylvania Avenue, N.W./Washington, D.C. 20006/Phone (202) 393-1700

March 11, 1978

Mr. Thomas De Cillis
Panel Administrator
OTC Review Panel on Dentifrices
and Dental Care Agents
Division of OTC Drug Evaluation
Bureau of Drugs
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

Dear Mr. De Cillis:

This submission is being made by the Subgroup on Fluoride Dentifrices for The Proprietary Association, 1700 Pennsylvania Avenue, Washington, D.C. 20006. The members of this Subgroup have been actively engaged in formulating the concept of laboratory profiles to establish the effectiveness of the three fluoride salts in conjunction with various dentifrice abrasives. At the 40th meeting of the OTC Panel on Dentifrices and Dental Care Agents, The Proprietary Association fluoride dentifrice profile charts were reviewed and accepted with modifications.

This submission is in response to the Panel's request for profile test methodology and for clarification on the use and the availability of clinical referenced fluoride dentifrices. This submission contains:

1. Laboratory profiles for fluoride dentifrices as modified and accepted by the Panel at the 40th meeting.

2. Methods for each of the laboratory and biological tests.

3. The Subgroup's recommendations for the definitions of reference formulations for use as performance standards and the manner by which they can be made available.

Specific methods are given for the analyses required for each dentifrice system. In some instances, more than one method is provided for a particular test, thereby offering prospective manufacturers of fluoride dentifrices flexibility in choice of methodology to the greatest possible extent. It is understood that future changes in the analytical and biological test methods may be incorporated as amendments to the final monograph.

Concerning the definition of reference formulations for use as performance standards, this submission describes the manner proposed for making available fresh reference product to fulfill the function of performance standards. We believe that the most satisfactory and most readily available products for general use as performance standards would be the fluoride dentifrice formulations marketed by the manufacturers cited in this submission. While these products may not always be identical to the original clinically tested formulations, they will always have laboratory profiles equivalent to, or better than, those of the original clinically tested formulations. This procedure will ensure that the performance standards will always be formulations that have been tested directly against clinically evaluated formulations.

We are also aware that, at some time in the future, it may become impossible to replicate exactly a clinically tested formulation. This would occur, for example, if an ingredient in that formulation became unavailable. Should this occur, the performance standards should be redefined as equivalents of the clinically tested formulations through an amendment to the monograph.

A third footnote has been added to the laboratory profile for sodium monofluorophosphate dentifrices noting the use of any clinically tested sodium monofluorophosphate formulation as a performance standard.

We wish to point out a minor change in the chart of laboratory profiles. Data for the stannous fluoride silica formulations have been obtained with samples diluted 1:10 with water, and not 1:3 as written in the original chart. The Panel discussed and approved the 1:10 dilution but neglected to include it in the formal motion to accept the chart. We corrected this detail.

The Subgroup on Fluoride Dentifrices of The Proprietary Association will be glad to discuss this submission with the Panel.

Very truly yours,

Lewis P. Cancro

Lewis P. Cancro

Chairman
The Proprietary Association
Subgroup on Fluoride Dentifrices

LPC:amp

TABLE OF CONTENTS

)

STANNOUS FLUORIDE

DENTIFRICES

FLUORIDE SOURCE: SnF_2

ABRASIVES: Insoluble Sodium Metaphosphate
Silica
Calcium Pyrophosphate

ANALYTICAL
Theoretical Total Fluorine: 1000 ppm

Allowable Range of Total Fluorine: 900-1120 ppm

Soluble Fluoride, (Ionic)	Fresh	Aged Minimal Value	Dilution
Insoluble Sodium Metaphosphate	$\geq$ 600 ppm	$\geq$ 500 ppm	(1:10)
Silica	$\geq$ 600 ppm	$\geq$ 500 ppm	(1:10)
Calcium Pyrophosphate	$\geq$ 288 ppm	$\geq$ 108 (1)	(1:3)
Soluble Sn^{++}			
Insoluble Sodium Metaphosphate	$\geq$ 2000 ppm	Qualitatively Detectable	(1:10)
Silica	Qualitatively Detectable	Qualitatively Detectable	(1:10)
Calcium Pyrophosphate	$\geq$ 900 ppm	Qualitatively Detectable	(1:3)

pH		Dilution
Insoluble Sodium Metaphosphate	4.2-5.4	1:4
Silica	4.6-5.1	1:3
Calcium Pyrophosphate	4.4-5.1	1:3

BIOLOGICAL (Any two tests)(2)	Fresh	Aged Minimal Value
Enamel Solubility Reduction	Performance Standard	Performance Standard
Animal Caries Reduction	Performance Standard	Performance Standard
Fluoride Uptake by Enamel	Performance Standard	Performance Standard

STANNOUS FLUORIDE DENTIFRICES
FOOTNOTES

1) Value corresponds to that of aged product found clinically effective.

2) The performance standard for the formulation requires that the numerical score in the laboratory test shall be both (1) significantly different from the score for a placebo formulation, and (2) no lower than the score for the reference formulation at the 90% confidence level.

INDEX TO TEST METHODS *

STANNOUS FLUORIDE DENTIFRICES

	Abrasive		
	Insoluble Sodium Metaphosphate	Silica	Calcium Pyrophosphate
ANALYTICAL			
Total Fluorine	(1) (3) (4) (5)	(2)	(1)
Soluble Fluoride (Ionic)	(9)	(10)	(9)
Soluble Stannous Ion	(19)	(18)	(17)
pH	(31)	(30)	(30)
BIOLOGICAL			
Enamel Solubility Reduction	(33)	(34)	(33) (34)
Animal Caries Reduction	(37-39)	(37- 39)	(37-39)
Fluoride Uptake By Enamel	(40)	(41)	(40) (41)

* The test procedures are found in the sections labeled "Analytical Methods" and "Biological Methods." Where more than one method is indicated for a particular test, the manufacturer may use any one of these methods.

SODIUM MONOFLUOROPHOSPHATE

DENTIFRICES

SODIUM MONOFLUOROPHOSPHATE DENTIFRICES

FLUORIDE SOURCE: Na_2PO_3F

ABRASIVES

Sodium monofluorophosphate is sufficiently stable in dentifrice formulations to justify guidelines that may not be possible for dentifrices containing only sodium fluoride or stannous fluoride. Positive clinical results have been obtained using enough different abrasives in sodium monofluorophosphate dentifrices to conclude that the use of any commonly used abrasives, alone or in combination, will not interfere with clinical effectiveness. Although some abrasives in combination with sodium monofluorophosphate maintain higher levels of soluble fluoride longer than others, all have been shown to be clinically effective.

ANALYTICAL	
Theoretical Total Fluorine	1000 ppm
Allowable Range of Total Fluorine	850-1150 ppm

	Fresh	Aged Minimal Value	Dilution
Total Soluble Available Fluorine	$\geq$ 800 ppm	$\geq$ 600 ppm	(max. of 1:10)
Fluorine as soluble $PO_3F^{=}$ ion	$\geq$ 650 ppm	$\geq$ Half of total Soluble Available Fluorine (1)	(max. of 1:10)
Fluoride as soluble F^- ion	from 10 to 150 ppm	From 10 ppm to the Soluble $PO_3F^{=}$ Concentration	(max. of 1:10)

pH (Max. dilution 1:10)

Alumina/MFP 6.4-9.0, Calcium Carbonate/MFP 7.0-10.0, Calcium Pyrophosphate/MFP 5.0-5.4
Dicalcium Phosphate/MFP 6.5-7.8, Insoluble Sodium Metaphosphate /MFP 5.6-6.9, Silica/MFP 5.5- 7.4

BIOLOGICAL (Any Two Tests) (2)(3)	Fresh	Aged Minimal Value
Enamel Solubility Reduction	Performance Standard	Performance Standard
Animal Caries Reduction	Performance Standard	Performance Standard
Fluoride Uptake	Performance Standard	Performance Standard

SODIUM MONOFLUOROPHOSPHATE DENTIFRICES
FOOTNOTES

1) Soluble $PO_3F^{=}$ is derived either by direct analytical measurement, or by subtracting soluble ionic fluoride from total soluble available fluorine.

2) The performance standard for the formulation requires that the numerical score in the laboratory test shall be both (1) significantly different from the score for a placebo formulation, and (2) no lower than the score for the reference formulation at the 90% confidence level.

3) Any clinically tested sodium monofluorophosphate/abrasive formula can be used as the performance standard for any other sodium monofluorophosphate/abrasive formulation.

INDEX TO TEST METHODS*

SODIUM MONOFLUOROPHOSPHATE

DENTIFRICES

	Abrasive					
	Insoluble Sodium Metaphosphate	Dicalcium Phosphate	Alumina	Silica	Calcium Pyrophosphate	Calcium Carbonate (Chalk)
ANALYTICAL						
Total Fluorine	(4)(5)	(4)(5)	(1) (4) (5)	(3 - 5)	(4) (5)	(6 - 8)
Total Soluble Available Fluorine	(11-13)	(11-13)	(11 - 13)	(11-13)(16)	(11-13)	(13-15)
Fluorine as Soluble $PO_3F^{=}$ ion	(20-24)	(20-24)	(20 - 24)	(20 - 24)	(20-24)	(23)(24)
Fluoride as Soluble F^- Ion	(27)(28)	(27)(28)	(27)(28)	(25)(27)(28)	(27)(28)	(26)
pH	(32)	(32)	(32)	(30)(32)	(32)	(31)
BIOLOGICAL						
Enamel Solubility Reduction	(36)	(36)	(36)	(34)(36)	(36)	(34-36)
Animal Caries Reduction	(37-39)	(37-39)	(37-39)	(37-39)	(37-39)	(37-39)
Fluoride Uptake	(40-43)	(40-43)	(40-43)	(40-43)	(40-43)	(40-43)

* The test procedures are found in the sections labeled "Analytical Methods" and "Biological Methods". Where more than one method is indicated for a particular test, the manufacturer may use any one of these methods.

SODIUM FLUORIDE

DENTIFRICES

SODIUM FLUORIDE DENTIFRICES

FLUORIDE SOURCE: NaF

ABRASIVES: High-Beta-Phase Calcium Pyrophosphate

ANALYTICAL

Theoretical Total Fluorine:	1000 ppm
Allowable Range of Total Fluorine:	900-1150 ppm

Soluble Fluoride, (Ionic)	Fresh	Aged Minimal Value	Dilution
High-Beta-Phase Calcium Pyrophosphate	$\geq$ 648 ppm	$\geq$ 403 ppm	1:3

pH		Dilution
High-Beta-Phase Calcium Pyrophosphate	6.5-8.0	1:3

BIOLOGICAL (Any Two Tests)(1)

	Fresh	Aged Minimal Value
Enamel Solubility Reduction	Performance Standard	Performance Standard
Fluoride Uptake by Enamel	Performance Standard	Performance Standard
Animal Caries Reduction	Performance Standard	Performance Standard

SODIUM FLUORIDE DENTIFRICES
FOOTNOTE

1) The performance standard for the formulation requires that the numerical score in the laboratory test shall be both (1) significantly different from the score for a placebo formulation, and (2) no lower than the score for the reference formulation at the 90% confidence level.

SODIUM FLUORIDE

DENTIFRICES

SODIUM FLUORIDE DENTIFRICES

FLUORIDE SOURCE: NaF

ABRASIVES High-Beta-Phase Calcium Pyrophosphate

ANALYTICAL

Theoretical Total Fluorine:	1000 ppm
Allowable Range of Total Fluorine:	900-1150 ppm

Soluble Fluoride, (Ionic)	Fresh	Aged Minimal Value	Dilution
High-Beta-Phase Calcium Pyrophosphate	$\geq$ 648 ppm	$\geq$ 403 ppm	1:3

pH		Dilution
High-Beta-Phase Calcium Pyrophosphate	6.5-8.0	1:3

BIOLOGICAL (Any Two Tests)(1)

	Fresh	Aged Minimal Value
Enamel Solubility Reduction	Performance Standard	Performance Standard
Fluoride Uptake by Enamel	Performance Standard	Performance Standard
Animal Caries Reduction	Performance Standard	Performance Standard

SODIUM FLUORIDE DENTIFRICES
FOOTNOTE

1) The performance standard for the formulation requires that the numerical score in the laboratory test shall be both (1) significantly different from the score for a placebo formulation, and (2) no lower than the score for the reference formulation at the 90% confidence level.

INDEX TO TEST METHODS*

SODIUM FLUORIDE DENTIFRICES

	Abrasive
	Calcium Pyrophosphate
ANALYTICAL	
Total Fluorine	(1)
Soluble Fluoride (Ionic)	(29)
pH	(30)
BIOLOGICAL	
Enamel Solubility Reduction	(33)
Animal Caries Reduction	(37- 39)
Fluoride Uptake By Enamel	(40)

*The test procedures are found in the sections labeled "Analytical Methods" and "Biological Methods." Where more than one method is indicated for a particular test, the manufacturer may use any one of these methods.

REFERENCE
FORMULATIONS

REFERENCE FORMULATIONS FOR PERFORMANCE STANDARDS

The following reference formulations are available from the manufacturers listed.

Stannous fluoride-silica	Lever Brothers Co.
Stannous fluoride-calcium pyrophosphate	Procter & Gamble Co.
Stannous fluoride-insoluble sodium metaphosphate	(See footnote*)
Sodium monofluorophosphate-insoluble sodium metaphosphate	Colgate-Palmolive Co.
Sodium monofluorophosphate-calcium carbonate (chalk)	Beecham Products
Sodium fluoride-high beta phase calcium pyrophosphate	Procter & Gamble Co.

Requests will be filled in an orderly manner within a reasonable time and at a reasonable cost.

Beecham Products
Western Hemisphere Research
1500 Littleton Road
Parsippany, New Jersey 07054

Colgate-Palmolive Company
Research and Development Dept.
909 River Road
Piscataway, New Jersey 08854

Lever Brothers Company
Research Center
45 River Road
Edgewater, New Jersey 07020

The Procter & Gamble Company
Toilet Goods Division
6110 Center Hill Road
Cincinnati, Ohio 45224

* The manufacturers listed above do not currently manufacture dentifrices based on insoluble sodium metaphosphate - stannous fluoride. However, copies of the clinically tested formulas (unflavored) and appropriate manufacturing procedures may be obtained from the Lever Brothers Company and/or the Colgate-Palmolive Company.

ANALYTICAL METHODS

ANALYTICAL TEST METHODS

TEST METHOD #1

Title: Determination of Total Fluorine in Stannous Fluoride or Sodium Fluoride Toothpastes by Fluoride Electrode

Recommended for the Following Systems:

a. Stannous Fluoride - calcium pyrophosphate

b. Stannous Fluoride - insoluble sodium metaphosphate

c. Sodium Fluoride - high-beta-phase calcium pyrophosphate

Test Method #1

Determination of Total Fluorine in Stannous Fluoride or Sodium Fluoride Toothpastes by Fluoride Electrode

Principle and Scope

This method measures total fluoride as % F^- in SnF_2 or NaF dentifrices by direct potentiometry. The dentifrice sample is slurried with EDTA/THAM solution under standardized conditions to solubilize the added fluoride. The emf developed by an Orion fluoride electrode in the toothpaste slurry, when the millivolt meter is standardized as described herein, is related to total fluoride (%F^-) in he paste by a calibration curve. This method is accurate for samples 0 - 12 months old.

Reagents	Suggested Type or Source (equivalent items may be used)
Sodium hydroxide solution	Approximately 5 N
Sodium fluoride	Baker's Analyzed Reagent, 99.5% minimum purity
(Ethylenedinitrilo) tetraacetic acid, disodium salt ($Na_2EDTA \cdot 2H_2O$)	ACS Grade
Distilled deionized water	
2-Amino-2-(hydroxymethyl)-1,3-propanediol (THAM)	Matheson, Coleman & Bell (m.p. 170-171°C.)

Apparatus

Pipets	5-ml to 50-ml
Magnetic stirrer	Labline "Magna-Stir"
Stirring bars	Teflon-coated, 7/8-inch
Polyethylene bottles	500-ml, 1000-ml
Volumetric flasks	100-ml, 500-ml, 1000-ml
Expanded-scale pH meter	Beckman Expandomatic
Fluoride-specific ion electrode (see Note 1)	Model No. 94-09-00, Orion Research, Inc. 11 Blackstone Street, Cambridge, Massachusetts 02139
Single-junction reference electrode	Model No. 90-01, Orion Research, Inc.
Electrode holder	Model No. 90-00-01, Orion Research, Inc.
Graph paper	Codex Brand No. 31,286 (two-cycle semi-log graph paper)
Plastic beakers	100-ml disposable
Watch glasses	75-mm

0.2 M EDTA/0.2 M THAM Solution (pH = 8.0)

Weigh 74.4 g disodium EDTA into a 1000 ml beaker. Add 24.2 g THAM (2-amino-2-(hydroxymethyl)-1,3-propanediol) and about 800 ml of hot distilled water (90-100°C.). Insert a stirrer or stirring bar. Insert the glass-calomel elctrode pair (standardized with pH = 7.0 buffer) and adjust the pH meter temperature control to the temperature of the solution. Slowly add 5 N NaOH solution until the pH of the solution is 7.5-8.0. Stir until the solids are all in solution and cool. Adjust the pH to exactly 8.0 by the addition of more NaOH as necessary. Transfer to a 1000-ml volumetric flask, rinse out the beaker several times with small portions of distilled water and add the rinsings to the flask, and finally dilute to the mark with distilled water and mix well.

Sodium Fluoride Stock Solution (0.03 mg F^-/ml):

Dry about 1 g of pure sodium fluoride at 105°C. for three hours and allow to cool in a desiccator. Weigh 0.3316 g of the dried reagent, dissolve in distilled deionized water, and transfer to a 500-ml volumetric flask. Dilute to volume and mix well. Transfer 50 ml of this solution to a second 500-ml volumetric flask, dilute to volume with distilled deionized water, and mix. This is the NaF stock solution, containing 0.03 mg F^-/ml. Store in a polyethylene bottle.

Standard Calibration Solutions

Prepare standard calibration solutions by carefully measuring the appropriate quantities of NaF stock solution (0.03 mg F^-/ml), EDTA/THAM solution, and water into 100-ml volumetric flasks as specified in the table below:

Solution	Volumetric	mg F^-/ml	ml NaF Stock Solution	ml EDTA/THAM	ml H_2O
1	100 ml	.0015	5	50	to volume
2	100 ml	.0060	20	50	to volume
3	100 ml	.0090	30	50	to volume
4	100 ml	.0120	40	50	to volume
5	100 ml	.0150	50	50	-

Temperature Control of Solutions and Slurries

In order to prevent the standardizing solutions and toothpaste slurries from being heated by the magnetic stirrer during the potential readings, a section of cardboard should be placed on the stirrer and the beaker set on the cardboard. The potential of each solution and slurry should be measured at room temperature (70-80°F).

Standardizing the Potential Scale of pH Meter

The pH meter should be used in the expanded scale and -mv modes. The potential range necessary for fluoride activity measurements for this application is 0 to -200 mv.

The scale of the meter is standardized by adjusting it to read -100 mv (the center of the mv scale) when the fluoride and reference electrodes are in contact with the 0.015 mg F^-/ml solution. (See Note 2) This standardizing procedure should be performed periodically (as noted below) in conjunction with emf measurements on both calibrating slurries and on test sample slurries to insure accuracy and reproducibility of results.

Before the electrodes are removed from a solution, the "stand-by" button on the meter must be pressed.

Response Check of Orion Fluoride-Specific Ion Electrode

The response of the fluoride electrode must be checked each time it is used to establish the potential response versus concentration of free fluoride. (This is to verify the slope and linearity of the electrode's response.)

The Orion single-junction reference electrode should be used as the reference electrode. (See Note 1.) The following solutions are necessary and sufficient for checking the response of the electrode:

Standard Calibration Solution #1 0.0015 mg F^-/ml

Standard Calibration Solution #5 0.015 mg F^-/ml

These can be prepared as described above. The electrodes are first put into Standard Calibration Solution #5 and the potential adjusted to read -100 mv (estimated to the nearest 0.1 mv. See "Standardizing the Potential Scale of the pH Meter" above.) Solutions #1 and #5 should then be read in that order; the potential of #5 should not drift more than $\pm$ 1 mv and should be readjusted to -100 mv in any case each time it is checked. The solutions should be stirred during the emf measurement, but not so vigourously as to allow bubbles to form and touch the fluoride electrode membrane crystal. At least one minute should be allowed for the electrode to reach a steady potential. The potential difference between Solutions #1 and #5 should be 59 $\pm$ 1 mv. If the electrode does not behave in this manner, it should be replaced by a new electrode.

Preparation of Calibration Curve

In order to determine fluoride levels in the toothpaste directly from the emf measurements of slurries as read on the pH meter, it is necessary to have a calibration curve for the electrode which converts millivolts to mg F^-/ml of slurry.

The calibration curve is prepared by spiking EDTA/THAM solutions with known concentrations of fluoride (see preparation of standard calibration solutions above) and plotting mg F^-/ml of solution (or slurry) vs. millivolts on semi-log graph paper. Before making emf measurements on the calibration solutions, the meter should be adjusted to read -100 mv when the electrodes are equilibrated in Calibration Solution #5 (0.015 mg F^-/ml). The potential adjustment with Calibration Solution#5 should be made after one minute of stirring to allow the electrode to reach a steady potential.

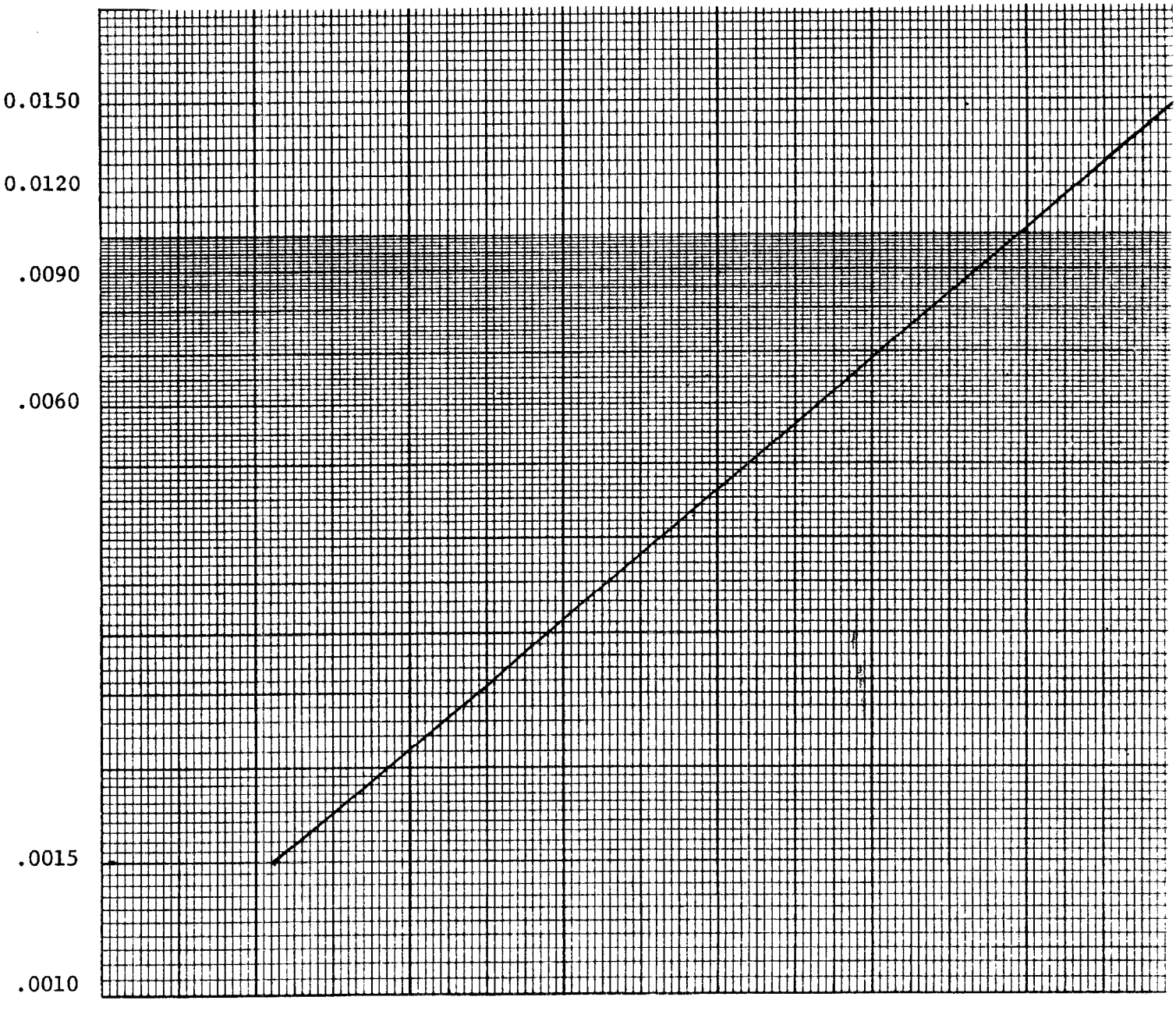
The electrodes should then be rinsed thoroughly with distilled water, especially the membrane crystal of the fluoride electrode, and gently wiped dry with absorbent tissue. The potential of another calibration solution is then measured after one minute of equilibration. The potential should be read to the nearest 0.1 mv and that value recorded. The electrodes are then rinsed and dried and the potential of Calibration Solution #5 remeasured. If the potential is more than $\pm$ 1 mv from -100 mv, the potential should be readjusted to -100 mv and the potential of the calibration solution remeasured. The emf's of the other calibration solutions are measured and recorded in the same way. (The technique of indexing the meter at -100 mv with a standardizing solution makes it possible to use the same calibration curve day after day and maximizes the precision and accuracy of the direct potentiometric electrode method.)

The sample calibration curve shown on the following page was prepared from the following data, generated by the method described above.

Calibration Solutions mg F^-/ml	Millivolts (from pH meter)
0.0015	- 41.2
0.0060	- 76.7
0.0090	- 87.0
0.0120	- 94.6
0.0150	-100.0

Each laboratory should generate its own calibration curve as described above, although all curves should appear similar to the sample curve shown.

CALIBRATION CURVE FOR TOTAL FLUORIDE



Preparation of Toothpaste Slurries

The analysis for total fluoride of the toothpaste is performed on slurries prepared in the manner described below.

Weigh 1 g of toothpaste to the nearest 1.0 mg into a 100-ml disposable plastic beaker. Disperse the toothpaste in 50 ml of EDTA/THAM solution, add a magnetic stirring bar and slurry the mixture on a magnetic stirrer for 20 minutes. It is important that all of the toothpaste sample is thoroughly dispersed in the slurry, and it may be necessary to move the beaker "off center" on the magnetic stirrer so that the stirring bar agitates all of the paste in the beaker. Transfer quantitatively to a 100-ml volumetric flask using distilled water to rinse out the beaker. Fill to the mark with distilled water, using a drop or two of alcohol to kill the foam at the meniscus. Mix well.

Test Samples

The total fluoride level in a sample of toothpaste is determined by preparing a slurry of the toothpaste as described above, and measuring the potential of the test slurry. Before making emf measurements on the test sample, the meter should be adjusted to read -100 mv (See Note 2) when the electrodes are equilibrated in Calibration Solution #5 (0.015 mg F^-/ml). The potential adjustment with the calibration solution should be made after one minute of stirring to allow the electrode to reach a steady potential. The procedure for test samples is then identical to that described above for the preparation of the calibration curve (See Note 3). The concentration of F^- as mg F^-/ml in the 100-ml slurry is ready from the calibration curve and converted to % F^- in the toothpaste (not the slurry) by the following equation:

$$\%F^- = \frac{\text{mg } F^-\text{/ml (in slurry) x 100 ml x 100}}{\text{wt. of toothpaste sample in mg}}$$

For example, if the slurry prepared from exactly 1.000 g toothpaste produced a reading on the pH meter of -90 mv, the $\%F^-$ in the toothpaste would be calculated as:

$$\%F^- = \frac{0.0102 \text{ (from the calibration curve) x 100 x 100}}{1000}$$

$\%F^-$ in toothpaste = 0.102%

It is very important that the response of the electrode be checked every day and that the scale of the pH meter be standardized frequently while samples are being tested (preferably before every sample emf is read).

Notes:

1. An Orion Fluoride-Specific Ion Combination Electrode, Model No. 96-09 may be used to replace the fluoride-specific ion electrode and single-junction reference electrode listed in the "Apparatus" section of the method.

2. Some pH meters cannot be adjusted to read -100.0 mv. The choice of -100 mv was arbitrarily chosen because it is midpoint on the scale; however, any other initial starting potential may be used if -100 mv is not attainable on your pH meter (-90 mv is suggested). The important point is that the pH meter be adjusted to this same initial mv reading prior to every reading that is taken on a calibration standard solution or sample test slurry.

3. The potential should be read to the nearest 0.5 mv and that value recorded. The electrodes should then be thoroughly washed with distilled water, especially the membrane crystal of the fluoride electrode. The electrodes are then gently wiped dry with absorbent tissue. The calibrating solution is then remeasured. If the potential is more than $\pm$ 1 mv difference from -100 mv, it should be adjusted to -100 mv and the potential of the supernatant remeasured.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #2

DETERMINATION OF TOTAL FLUORINE

Recommended for the following systems:

a. Stannous fluoride and silica abrasive.

TEST METHOD #2

DETERMINATION OF TOTAL FLUORINE

Principle

This method is specifically for silica-based toothpastes containing stannous fluoride.

The toothpaste sample is solubilized by treating with hot dilute sodium hydroxide solution and then neutralized with acetic acid.

The fluoride content of this solution is determined by the potentiometric technique of multiple standard addition. In this technique incremental volumes of a standard fluoride solution are added to the sample solution. The potentials developed at a fluoride sensitive electrode vs. a suitable reference electrode are observed at each step. These stepwise changes in potential, measured in millivolts, are related to the original fluoride concentration of the sample solution by a computation derived from the equation (*).

Equation (1)

$$C_x = \frac{C_s}{10^{\frac{\Delta E}{\text{slope}}}\left[1 + \frac{V_x}{V_s}\right] - \frac{V_x}{V_s}}$$

Term C_x is the unknown concentration, Cs is the fluoride concentration in the standard solution, ΔE is the potential change caused by the addition of the known increment of fluoride solution, "slope" is the slope of the Nernst equation, V_s is the volume of fluoride standard added, and V_x is the original volume of sample solution.

Apparatus

Corning Digital 112 Research pH/mV Meter.
Orion Fluoride Specific-Ion-Sensitive Electrode 94-09A.
Orion Single-Junction Reference Electrode 90-01 and a supply of Orion Reference Electrode Filling Solution 90-00-01. DO NOT USE ANY OTHER FILLING SOLUTION!
Magnetic Stirring Apparatus.
Buret, 25-ml meeting Class A specifications.
Volumetric Flasks, 250-ml and 1000-ml sizes.
Pipets, 10-ml, 20-ml, 25-ml sizes, Class A.
Heath EUA-20-12 pH/mV Test Box.

Reagents

Sodium Hydroxide Pellets, ACS Reagent Grade. Prepare a liter of Sodium Hydroxide Solution, 10% w/v in distilled water. Store in a polyethylene bottle.
Glacial Acetic Acid, ACS Reagent Grade. Prepare 2 liters of Acetic Acid Solution, 10% v/v in distilled water.

*Karlberg, B., Anal. Chem. 43 1911-1913 (1971).

Phenolphthalein Indicator Solution, prepare as in A.M. 0.020 for laboratory use.
Sodium Fluoride, ACS Reagent Grade, preferably Fisher Certified S-299. Prepare the solution shown below.
Master Fluoride Solution (2500 ppm w/v Fluoride, pH 5).
Dissolve 5.526 grams of reagent grade ACS-specification sodium fluoride in water, add 40 ml of 10% w/v sodium hydroxide solution, then add 80 ml of 10% v/v acetic acid solution and mix. Transfer the mixture quantitatively to a 1000-ml volumetric flask. Make the contents of the flask to the mark with water near 25°C and mix thoroughly.
Transfer the solution to a polyethylene bottle for storage.

Procedure

A few instrumental preliminaries are needed. The fluoride electrode is preferably stored dry, covered with a cap to protect the fragile crystal membrane. The reference electrode is also stored with a protective cap to prevent evaporation. Remove these caps, inspect both electrodes for obvious mechanical damage, and if none is evident, immerse the pair in stirred distilled water. Refill the reference electrode if necessary, using only the Orion filling solution.

The Corning meter may be left on permanently. If there is any suspicion that the meter is not functioning properly, use the Heath test box to check the meter, remembering that the Corning meter is more accurate than the test source, and this procedure will only detect gross error.

Preheat a hot plate while samples are being weighed. An 8" Chromalox 1500-watt hot plate is recommended for fastest warmup time.

Tare a 300-ml Pyrex beaker. Accurately weigh into it by difference a 2.5 to 3.0-gram sample of toothpaste. Add 10 ml of 10% w/v sodium hydroxide solution from a pipet. Add 50 ml of water from a graduate.

The next operation is slightly hazardous, and appropriate eye protection is demanded. Beaker tongs and gloves must also be used. Using a 10-inch glass stirring rod for constant stirring to avoid violent bumping, heat the contents of the beaker to a rolling boil for approximately a one-minute boiling period. Vigorous foaming will occur, and the mixture will become almost clear. Without interrupting the stirring, lift the beaker from the hot plate until the foaming subsides. Stop stirring when foaming stops, then let the mixture cool for approximately ten minutes.

Quantitatively transfer the mixture from the beaker to a 250-ml volumetric flask without using excessively large amounts of water. A small quantity (several drops) of phenolphthalein indicator solution may be added to the flask if desired. Pipet 20 ml of 10% v/v acetic acid solution into the flask, swirling it gently as the addition is made. Neutralization will occur before the entire quantity of acetic acid solution has been added. Make the contents of the flask to the mark with water and mix. Invert the beaker on a towel for a few minutes to drain.

Without using any additional water, transfer the contents of the flask back into the original beaker, draining the flask well by use of a small ring clamp. A small transfer error is involved here, but its size is trivial. Clean the

volumetric flask before the film of solution on its surface has dried, or it will be difficult to clean. Cover the beaker with a watch glass or Parafilm until the determination can be completed. Slow precipitation of hydrous silica may occur. Wait at least ½ hour to allow nucleation of of hydrous silica to begin. The next operation may be performed immediately or within 24 hours irrespective of the sample appearance. Overnight standing ordinarily results in copious precipitation of hydrous silica, but this has no noticeable effect on the results.

The multiple additions procedure requires several precautions. The sample temperature must be within a few degrees of room temperature, and most stirrers require thermal insulation to keep the sample from heating. A square of plastic foam covering the stirrer top is advised. The electrode immersion depth should be fixed, and the stirring rate should be fixed but not vigorous enough to create a vortex. The electrodes should be wiped gently with a tissue before every immersion. If a blank is to be analyzed because of suspected fluoride contamination, the blank must be the first analysis of the day, because the electrode has a definite "memory". Analysis of blanks is not necessary except when reagent lots are changed.

Record the mV reading of the electrode pair in distilled water. Immerse the electrodes in the stirred sample solution, and follow the timing schedule below. Precise timing is not required, but these minimum times must be observed.

(1) Record the initial potential after 5 minutes.
(Exception: Wait ten minutes if this is the first sample of the day.)

(2) Add 1.00 ml of 2500 ppm fluoride master solution from a buret, and record the potential 2 minutes after the addition is complete.

(3) Four additional 1.00-ml volumes of 2500 ppm fluoride master solution are added next, so for buret readings of 2.00, 3.00, 4.00, and 5.00 record the potential 1 minute after the addition is complete.

(4) After each sample, rinse the electrodes briefly with distilled water, then return them to stirred distilled water until the reading is approximately 120 mV higher than the initial reading from a fluoride-containing toothpaste. Record these readings also. A properly functioning fluoride electrode should attain these responses in 2 minutes or less. It is not necessary or desirable to wash the electrode exhaustively with water between analyses, because of the "memory" effect.

Computations

Fluoride is calculated in accordance with Equation (1), above. Five values of this equation are computed for each of five additions of a standard fluoride solution. The mean of these and its estimated standard deviation are calculated, too.

C_x is the unknown fluoride concentration of the sample solution.

C_s is the fluoride concentration of the standard solution.

ΔE_1 is the incremental potential change in millivolts observed upon addition of the first volume of standard fluoride.

V_x is the original volume of the sample solution in milliliters.

V_s is the totally added volume of the standard fluoride solution in milliliters.

A value of -59.16 millivolts per decade change of fluoride activity may be assumed. It is convenient to express fluoride concentrations in micrograms F per milliliter. Results are then recomputed to the paste basis.

(The equation is not as formidable as it appears to be. If a fluoride electrode has a negative logarithmic response to fluoride activity, it is intuitively obvious that fluoride activity will be an inverse antilogarithmic function of electrode potential. The remaining terms in the equation are volume corrections).

A volume-corrected Gram Function (2) is also calculated for each of the five additions. This antilogarithmic function provides additional confidence in the performance of the method.

The true slope and standard potential of the electrode system can be approximated from a calibration line by nonlinear regression analysis of calibration data of the form $y = a + b \log_{10} x$. However, these are best determined by the calculation of Brand and Rechnitz (3), which also provides an independent estimate of fluoride concentration. This computation is a formidable numerical analysis routine demanding the facilities of a computer. The earlier computations are readily programmable.

Example

Assume a 2.500 gram sample of dentifrice containing its target level of 1000 µg F per gram of paste, or a total of 2500 µg F. This 2500 µg quantity of F dissolved in 250 ml total volume givesa fluoride concentration of 10.000 micrograms per milliliter. Now add five separate 1.00-ml increments of a standard fluoride solution with 2500 µg F/ml. Observe the response of a fluoride electrode with perfect slope (-59.16 mV) and a standard potential of -180 mV at 1 molar F activity. The following conditions should prevail:

Ml added	µg F present	Volume, ml	ppm F	pF Molarity	E in Millivolts
None	2500	250	10.00	3.27875	13.9711
1.00	5000	251	19.9203	2.97946	-3.7353
2.00	7500	252	29.7619	2.80509	-14.0507
3.00	10,000	253	39.5257	2.68187	-21.3403
4.00	12,500	254	49.2126	2.58668	-26.9722
5.00	15,000	255	58.8235	2.5092	-31.5556

(1) Karlberg, B. Anal. Chem. 43 1911-1913 (1971).
(2) Gran, G. Analyst 77 661-671 (1952).
(3) Brand, M.J.D., and Rechnitz, G.A. Anal. Chem. 42 1172-1177 (1970).

Ml added	Delta EomV	Volume-Corrected Gram Function	Computed F µg/ml of Original Solution by Equation (1)
None	(Initial)	-	-
1.00	-17.71	2.0003	9.9972
2.00	-28.02	2.9998	10.001
3.00	-35.31	3.9998	10.0007
4.00	-40.94	4.9994	10.0016
5.00	-45.53	6.0008	9.9984

The last column obviously confirms the target level of 1000 µg F per gram on the original sample of dentifrice in this example.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #3

DETERMINATION OF TOTAL FLUORINE

Recommended for the following systems:

a. Sodium monofluorophosphate and insoluble sodium metaphosphate abrasive.

TEST METHOD #3

DETERMINATION OF TOTAL FLUORINE

A. TOTAL FLUORINE

Principle

The sample is acidified with perchloric acid, whereby volatile hydrogen fluoride is formed. The hydrogen fluoride is then trapped in the lid of a Petri dish, coated on the inside with a known amount of sodium hydroxide. The fluoride concentration in the trapping cover is determined with a fluoride-sensitive electrode. The potential produced across these electrodes is measured on the millivolt scale of a pH meter, and compared to a standard graph constructed on semi-log paper. This method is applied to the determination of total fluoride in toothpastes containing sodium monofluorophosphate (MFP. It is not applicable to toothpastes containing calcium carbonate as the abrasive.)

Apparatus

Corning Digital 112 Research pH/mV Meter.
Orion Fluoride Specific-Ion-Sensitive Electrode 94-09A.
Orion Single-Junction Reference Electrode 90-01.
Magnetic stirring apparatus.
Volumetric flasks, 25-ml, 50-ml, 1000-ml sizes.
Pipets, 1-ml, 2-ml, 3-ml, 5-ml, 8-ml and 25-ml sizes.
Polyethylene beakers, 100-ml.
Petri dishes, 60 x 15 mm style.
Polyethylene bottles, 50-ml.
Semi-log graph paper, 1 cycle.
Oven
Desiccator
Centrifuge, Servall Type SP, Ivan Sorvall, Inc.
Centrifuge tubes, round bottom, polypropylene, 50-ml capacity.
Centrifuge tubes closure to fit 50-ml centrifuge tubes.
Buret, 50-ml.

Reagents

Sodium hydroxide pellets, ACS Reagent Grade. Prepare 100 ml of 0.25N of a 3A alcoholic sodium hydroxide solution.

Sodium fluoride, ACS Reagent Grade. Prepare the solution shown below: Weigh accurately 2.2105 grams of reagent grade sodium fluoride. Dissolve and dilute to 1 litre in water (1000 ug/ml F^- standard). Pipet 10 ml of 1000 ug/ml fluoride standard into a 1000-ml volumetric flask, dilute to volume with water and mix thoroughly. (10 ug/ml F^- standard). Transfer the solution to a polyethylene bottle for storage.

Perchloric acid, 70% Reagent Grade.

Sodium citrate dihydrate, Reagent Grade.

Sodium chloride, Reagent Grade.

Glacial acetic acid, Reagent Grade.

Buffer solution: Dissolve 147 grams sodium citrate dihydrate and 58 grams sodium chloride in 1000 ml water. Add 60 ml glacial acetic acid. Mix by stirring. Adjust with sodium hydroxide (approx. 10% solution) to pH 5.5. Cool the solution then make up to 2 liters with water, and mix well. Store in a polyethylene bottle.

Calcium chloride - anhydrous, Reagent Grade.

Preparation of Standard Curve

Connect the lead of the fluoride electrode to the shielded input jack of the pH meter. Fill the reference electrode with the special filling solution supplied by Orion (No. 90-00-01). Do not use any other filling solution. Connect the lead of the reference electrode to the reference input jack of the pH meter. Turn the meter onto STANDBY function and place the electrodes in distilled water for about 15 minutes to allow them to equilibrate. Pipet 1 ml of 10 ug/ml fluoride standard and 25 ml buffer solution into a 50 ml volumetric flask (Note), dilute to volume with water, and mix well. Without using any additional water, transfer the contents of the flask into a clean 100 ml plastic beaker, draining the flask well. Carefully blot dry the electrodes with Kleenex tissues. Immerse the electrode tips in the buffered fluoride standard. Ensure that there are no air bubbles adhering to the electrode surface. Drop the stirring bar between them and stir at a moderate rate. Leave for 5-10 minutes until a constant potential is produced. Record the millivoltage. Remove the electrodes, rinse with distilled water and dry with Kleenex tissues. Determine the millivolt readings on the other standard solutions in a similar manner by replacing 1 ml of 10 ug/ml fluoride standard with 2 ml, 3 ml, 5 ml and 8 ml of 10 ug/ml fluoride standard respectively. Plot the calibration graph on semi-log paper with the millivolt reading on the linear abscissa and concentration on the logarithmic ordinate.

Preparation of Diffusion Dishes

Cover the inside of the Petri dish cover with a known amount of sodium hydroxide by placing 0.3 ml of 0.25N ethanolic sodium hydroxide in the lid, covering the whole surface by a gentle rotating movement. Evaporate the alcohol in a desiccator containing calcium chloride to leave a perfectly

regular adherent layer. If the layer is not regular do not use the dish, but prepare a fresh one. Dishes may be prepared in advance, but must be stored in a desiccator containing anhydrous calcium chloride.

Procedure

Weigh about 2 grams of paste, to an accuracy of 1 mg into a 50-ml beaker. Slurry the sample with about 20 ml of distilled water, adding 1-2 ml of water at a time and stirring with a small glass rod. Transfer the slurry quantitatively to a 100-ml volumetric flask with distilled water, dilute to volume with additional water, and put the small stirring bar in the flask. Stir for a few minutes then pipet 2 ml of sample solution into the lower part of the Petri dish. Add 4 ml of 70% perchloric acid and cover immediately with the prepared lid. Heat in an oven at 60°C overnight. Remove the dish from the oven and immediately remove the cover. Wash the inside of the cover about five times with a few ml of water, catching the wash in a 50-ml volumetric flask (Note) containing 25 ml of buffer. Make up to volume with water. Read the potential in millivolts, following the procedure used for the standard solutions.

Note: A glass volumetric flask may be used if the solution is transferred to a plastic container after diluting to volume.

Calculations

Determine the fluoride concentrations in the sample solution by referring to the standard curve.

$$\% \text{ Total Fluoride} = \frac{F \times 100 \times 100}{W \times 2 \times 10^6}$$

$$= \frac{F}{W \times 200}$$

F = µg of fluoride read from the standard curve.

W = Weight of sample in grams.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #4

TITLE: DETERMINATION OF TOTAL FLUORINE

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/ Silica

e. Sodium Monoflorophosphate/Calcium Pyrophosphate

TEST METHOD #4

DETERMINATION OF TOTAL FLUORINE

PRINCIPLE

In the automated method for total fluoride, the sample is diluted to volume in an acid solution, an aliquot is centrifuged and a portion of the supernatant liquid is introduced into the AutoAnalyzer. For calibration, the standard contains known amounts of fluoride plus the dental cream formulation placebo being analyzed.

APPARATUS REQUIRED

Technicon AutoAnalyzer equipped with the following modules:

1. Sampler II with a 20, 1-2 cam and 4.0 or larger ml sample cups.
2. Proportioning Pump. Either the standard or Pump II may be used.
3. High temperature heating bath set at 155°C to 165°C and equipped with a 15 ft. teflon coil. See Note A.
4. Microdistillation unit (Boyce Thompson Method, Technicon Symposia 1965, p. 270).
5. Vacuum pump, adjustable, maintained at 5 or 6 in. of Mercury.
6. Colorimeter equipped with a 15 mm flow cell and 619 range mu filters.
7. Recorder, single pen. Chart speed 2.54 cm per 200 seconds.

The Boyce Thompson microdistillation apparatus is as described by Mandl, Weinstein, Jacobson, McCune and Hitchcock at the Technicon Symposium, "Automation in Analytical Chemistry," N.Y., N.Y., September 8, 1965. The manifold is as designed by Phil Hughes of the Jeffersonville, Ind., plant of Colgate-Palmolive Co.

The modules are assembled as shown in attached flow diagram.

The waste line discharge is highly acidic and it must be neutralized before being discarded in drain pipes.

REAGENTS REQUIRED

1. Alizarin Complexone: Available from K and K Laboratories, Inc., Plainview, N.Y. and Hollywood, Calif. Suspend 0.960 grams of Alizarin Complexone in 100 ml water contained in a 250 ml volumetric flask. Add 2 ml of Ammonium Hydroxide and shake until the dyestuff is dissolved. Add 2 ml of Acetic Acid, dilute to volume with water and mix well. Store the solution in a refrigerator.

2. Lanthanum Nitrate: Available from Fisher Scientific Co., Catalog No. L-10. Weigh 1.08 grams of Lanthanum Nitrate and transfer to a 250 ml volumetric flask. Dissolve in water, dilute to volume with water and mix well.

3. Sodium Acetate Buffer (pH 4.0): Dissolve 60 grams of Sodium Acetate Trihydrate in water. Transfer the solution to a 1 liter volumetric flask and add 100 ml of Acetic Acid. Dilute to volume with water and mix well.

4. Sulfuric Acid (60%): Carefully add 600 ml of Sulfuric Acid to about 400 ml of water contained in a 1 liter volumetric flask. Cool and mix well.

5. Working Reagent: Pipet 18 ml of Alizarin Complexone solution and 18 ml of Lanthanum Nitrate solution into a 500 ml volumetric flask. Using a graduate add 135 ml of Acetate Buffer solution, 90 ml of Acetone and 90 ml of t-Butyl Alcohol. Dilute to volume with water. Bubble nitrogen through the solution for 20 minutes.

6. Calcium Oxide, Low in Fluoride: Available from Fisher Scientific Co., Catalog No. C-117.

7. Perchloric Acid, 70-72%.

8. Standard Fluoride Solution: Weigh accurately 0.8842 g NaF (Baker Analyzed Reagent Grade) and transfer quantitatively to a 1000 ml volumetric flask. Dilute to volume with distilled H_2O. One milliliter of this solution equals 0.4 milligrams of Fluoride.

9. Placebo Standards: The placebo standards act as a blank and consist of the complete dental cream formulation except that the fluoride donor has been omitted. A 1.2 ± 0.1 grams of the placebo weighed on a torsion balance, is slurried with 60-80 ml of water in a small beaker and transferred to a 200 ml volumetric flask. A known amount of standard fluoride solution is added with a burette to the volumetric flask followed by the addition with a graduated cylinder of 20 ml of perchloric acid. The solution is then diluted to volume with distilled water and mixed on a magnetic stirrer. A portion of the solution is centrifuged and the clear supernatant liquid is the completed standard to be used in the AutoAnalyzer sampler cups. Suggested fluoride standards to be used for routine analysis are as follows:

The following standard fluoride solutions are made by dilution of the stock solution: 2·4, 2·8 3·2 3·6 mg F/500 ml.

	Placebo Fluoride Standards		
mg F/200 ml	1.44	1.28	1.12
ml of Std. Fluoride Soln (0.4 mg F/ml)	3.6	3.2	2.8
Wt. of Placebo	1.2	1.2	1.2
ml of 70% Perchloric Acid	20	20	20

PROCEDURE

(See Flow Diagram)

1. Accurately weigh (to the nearest milligram) 1.2 to 1.4 grams of sample into a 100 or 150 ml beaker.

2. Add 50 ml of water. Stir the mixture with a small glass rod until homogeneous. Wash off the glass rod with a small amount of water.

3. Add a stirring bar to the beaker and place on a magnetic stirrer. Mix well.

4. While mixing, add 20 ml of perchloric acid slowly. Continue mixing for 30 minutes.

5. Quantitatively transfer the contents to a 200 ml volumetric flask. Dilute to volume with water and mix well.

6. Centrifuge a portion of the solution from the volumetric flask until clear.

7. Transfer the clear supernatant liquid to the plastic sampler cup.

8. With the fluoride manifold on the proportioning pump and all the lines in water lower the roller head to start the instrument. Start the vacuum pump and adjust to 5 or 6 inches of Mercury. Keep the vacuum constant once the test is started.

9. Run water through the lines for a few minutes to check the flow. Dip all lines in their respective solutions.

10. Set the sampler table with the 20, 1-2 samples per hour cam and load the tray with 4.0 ml plastic cups.

11. The cups are filled alternately with standards and samples. Since each laboratory's needs will vary, no fixed procedure for this step can be made. It is recommended that the first 22 cups, which will approximate a one hour run, be filled as follows: See Note A.

Cups 1 - 4	1.12	Standard (mg F/200 ml)
Cups 5 - 10		Sample Solutions*
Cups 11 - 13	1.44	Standard (mg F/200 ml)
Cups 14 - 19		Sample Solutions*
Cups 20 - 22	1.28	Standard (mg F.200 ml)

*Two cups are filled for each sample and the average of the two analyses used in the calculation. Single analyses may be run once the instrument has been shown to yield consistent results.

12. The succeeding cups are filled alternately with samples (in duplicate), three 1.44 standards, samples (in duplicate), and three 1.12 standards.

13. Turn on the colorimeter and recorder.

14. When the baseline is established, set the absorbance at 0.01 and start sampling, making certain that the sampling probe dips well into the cups. It is essential that a steady bubble pattern of small bubbles be established after the working reagent is added. A steady baseline will be assurance of this.

15. After the last sample has been aspirated turn off the sampler and discard the cups.

16. After the last sample trace appears on the recorder chart, dip all the lines in water and flush the system.

17. After the instrument lines are washed for about 15 minutes, lift up the roller head, release the manifold and stop the vacuum pump.

18. Remove the recorder chart and record the absorbance values of each peak at the peak height. Ignore the first peak from the 1.12 standard.

CALCULATIONS

The calculations are performed on sets representing each hour of running time (steps 11 and 12).

1. Calculate the average absorbance of each standard by adding the absorbance values and dividing by three. The first cup peak must be ignored.

2. For each standard calculate the K value as follows:

$$K = \frac{\text{Milligrams F per 200 ml}}{\text{Average Absorbance of each Standard}}$$

3. Calculate the average K_{AV} value for each hour run by adding the standard K values and dividing by the number of standards represented. It is important to determine the K_{AV} for each hour of operation and to use the value obtained for calculation of results for samples included in that run. See Note B.

4. Calculate the Total Fluoride as follows:

$$\frac{(K_{AV} \times A) \times 100}{S} = \text{Total Fluoride as F}$$

Where K_{AV} = Average K value (calculation 3).
A = Average absorption of samples.
S = Sample weight in milligrams (step 1).

NOTES:

A. The indicated order in which the cups are filled is only given as an example and the samples and standards may be interchanged as desired.

B. It has been observed that the system may need to be standardized continuously. Thus, hourly averages of K for standardization are suggested. For instance, a run of 3 hours would require three average K values and each K value would be used in calculating the % fluoride content of the inclusive unknowns.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #5

TITLE: DETERMINATION OF TOTAL FLUORINE

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #5

DETERMINATION OF TOTAL FLUORINE

PRINCIPLE

The organic matter is removed by ashing in the presence of calcium oxide. The fluoride is distilled from a perchloric acid solution of the ashed residue and reacted in solution with Eriochrome Cyanine R and Zirconyl Chloride. The bleaching effect of the fluoride on the Zirconium lake is measured spectrophotometrically and the fluoride level is obtained by reference to a standard graph.

METHOD

APPARATUS REQUIRED

1. Steam Distillation Apparatus of borosilicate glass with band heater as shown in Figure I, LaPine Scientific Co., Irvington-On-Hudson, N. Y.

2. Glas-Col Heating Mantile, hemispherical to fit 2000 ml flask.

3. Variable Transformers (2) such as the "Powerstat" Type 116, Superior Electric Co., Bristol, Conn., or "Variac," Type V-5, General Radio Co., Cambridge, Mass. These transformers are available from most laboratory supply houses.

REAGENTS REQUIRED

Zirconyl Chloride Solution ($ZrOCl_2$)

Dissolve 0.265 gram of Zirconyl Chloride Octahydrate ($ZrOCL_2 \cdot 8H_2O$) in 50 ml of water. Add 700 ml of conc. Hydrochloric Acid and cautiously dilute to volume with water at room temperature in a 1000 ml volumetric flask. Mix well.

NOTE:

0.220 gram of Zirconyl Nitrate Dihydrate ($ZrO(NO_3)_2 \cdot 2H_2O$) may be used in place of Zirconyl Chloride Octahydrate.

Silver Perchlorate Solution ($AgClO_4$) 50%

Dissolve 250 grams of anhydrous Silver Perchlorate ($AgClO_4$) in 400 ml of water and dilute to 500 ml.

Eriochrome Cyanine R Solution

Dissolve 1.8 grams of Eriochrome Cyanine R (obtainable from Geigy Dyestuffs Div., Geigy Chemical Corp., N.Y. 8, N.Y.) in 100 ml of water and dilute to 1000 ml.

Sodium Hydroxide Solution (NaOH), 5%

Dissolve 5 grams of Sodium Hydroxide in 50 ml of water and dilute to 100 ml.

Calcium Oxide (CaO): the grade obtainable from Fisher Scientific Co., Catalog No. C-117 (0.005% Fluoride) is recommended.

Sodium Fluoride (NaF) Reagent Grade.

Perchloric Acid ($HClO_4$), 70%.

Hydrochloric Acid Solution (70 ml conc. HCl + 30 ml water) - Baker or Mallinckrodt HCl is recommended.

PREPARATION OF STANDARD CURVE

1. Accurately weigh 0.8842 gram of Sodium Fluoride and transfer to a 1000 ml volumetric flask. Add 500 ml of water and shake to dissolve the sample. Dilute to volume with water at room temperature and mix well. Store in a polyethylene bottle.

2. Pipet 10 ml of the sample solution from step 1 into a 1000 ml volumetric flask. Dilute to volume with water at room temperature and mix well. Each ml of this solution contains 0.00400 milligram (4 micrograms) of F. Store in a polyethylene bottle.

3. Using a buret, deliver 5.00 ml, 7.50 ml, 10.00 ml, 12.50 ml and 15.00 ml aliquots of the dilute solution from step 2 into separate 100 ml volumetric flasks. Add approximately 50 ml of water. Pipet 5 ml of Eriochrome Cyanine R solution and 5 ml of Zirconyl Chloride solution into each flask. Dilute to volume with water and mix well.

4. Using 1 cm cells in the Beckman DU Spectrophotometer, measure the absorbance of each solution from step 3 at 527.5 millimicrons. Use the reference solution as a blank. See step 14.

5. Using K&E 359-14L graph paper, plot the observed absorbance as the ordinate and milligrams of Fluoride as the abscissa. Draw the best possible straight line through the plotted points. Any points not falling on the line should be checked. A new standard curve must be prepared each time a new batch of dye or Zirconyl Chloride is used. The standard curve should be checked frequently.

SAMPLE ANALYSIS

1. Accurately weigh 1 ± 0.01 gram (to the nearest 0.1 milligram) of sample into a small platinum or nickel dish.

2. Weigh 1 ± 0.2 gram of Calcium Oxide and add about half of this weight to the sample in the dish. Mix well with a glass rod. Carefully wipe off the material clinging to the rod with a small piece of filter paper. Add the filter paper to the sample in the dish. Sprinkle the remaining Calcium Oxide over the sample.

3. Heat on an asbestos pad in an oven at 105°C to dryness. Char under a heating lamp and finally ash in a muffle furnace maintained at 580-620°C for 1 hour. At no time should the sample be permitted to ignite and burn with a flame.

4. Assemble the apparatus as shown in Figure 1 with the 1500 ml steam generator flask filled 2/3 with water. The apparatus should be cleaned once a week when in continuous use, by placing 5% NaOH solution in the distilling flask and steaming for 1/2 hour followed by thorough rinsing with water.

5. Cool the dish and contents to room temperature and quantitatively transfer the contents to the distilling flask with the aid of 50-75 ml of water. The total volume of water should not exceed 75 ml. Add approximately 0.5 gram of glass wool and spread evenly to cover the bottom of the flask.

6. Add 50 ml of 70% Perchloric Acid and 1 ml of 50% Silver Perchlorate solution to the sample solution in the distilling flask. The final volume in the distilling flask should not exceed 125 ml. Place a 500 ml volumetric flask containing a funnel under the distillate outlet.

7. Turn on the transformer connected to the heating mantle surrounding the steam generator and set at the highest dial reading (about 130). Leave the Hoffman clamp open until the water boils.

8. Turn on the transformer connected to the band heater and set the dial at 100.

9. When the temperature in the distilling flask is approximately 130°C and the water is boiling in the steam generator, steam is admitted to the distilling flask by closing the Hoffman clamp. The temperature in the distilling flask is maintained at 135° ± 2°C by temporarily turning off the transformer connected to the band heater if the temperature reaches 137°C and turning it on again if the temperature drops to 135°C. The installation of an automatic temperature control, known as the "Therm-O-Watch," to the band heater and thermometer of the distillation unit will allow the operator to perform other duties while the distillation is in progress. The "Therm-O-Watch" unit (Model S-6) is obtainable from Instruments for Research and Industry, 108 Franklin Avenue, Cheltenham, Pa.

10. Collect about 450 ml of distillate. Turn the unit off by opening the Hoffman clamp and shutting off both transformers.

11. Neutralize the distillate to litmus by the dropwise addition of 5% Sodium Hydroxide solution and dilute to volume with water at room temperature. Mix well.

12. Pipet a 20 ml aliquot of the solution from step 11 into a 100 ml volumetric flask. Add approximately 50 ml of water. Using micro burets, measure 5 ml of Eriochrome Cyanine R solution and 5 ml of Zirconyl Chloride solution into the flask. Dilute to volume and mix well.

13. Using 1 cm cells in the Beckman DU Spectrophotometer, measure the absorbance of the solution at 527.5 millimicrons versus a reference solution prepared as described in step 14.

14. The reference solution is prepared as follows: Measure 3.00 ml of Eriochrome Cyanine R solution into a 100 ml volumetric flask. Dilute to about 85 ml with water and add from a pipet 10 ml of 70/30 HCl solution. Dilute to volume and mix well. In some cases variations in reagents may result in an abnormal standard curve (Low Absorbance readings for each standard). In this case dilute the reference solution with a known and accurately measured volume of water so that the absorbance readings are on scale. The absorbance of the 5 ml standard should be between 0.4 and 0.5. The same reference solution used to prepare the standard curve must be used in analyzing samples.

15. From the absorbance, determine the milligrams of Fluoride by reference to the Standard Curve.

CALCULATION

1. $$\frac{(\text{mg F from Curve}) \times 100}{\text{Wt. of Sample} \times 1000 \text{ in Aliquot in grams step 12}} = \% \text{ Total Fluoride as F}$$

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #6

DETERMINATION OF TOTAL FLUORINE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #6

DETERMINATION OF TOTAL FLUORINE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

The objective of this method is to measure the total fluorine found in a sodium monofluorophosphate chalk dentifrice.

Reagents	Suggested Type or Source
n-Pentane 99%	Tridom Chemical or equivalent
Toluene 99%	MCB Chromatoquality
HCl Conc.	Baker Analyzed Reagent
Trimethylchlorosilane 99%	Tridom Chemical or equivalent
NaF	Baker Analyzed Reagent
Test tubes(screw-cap)	S.G.A.
Pipettes	S.G.A.

Apparatus	Suggested Type or Source
Gas Chromatograph Model 5710A with dual flame ionization detector	Hewlett-Packard

Column: 6 ft. 1/8" OD stainless steel packed with 25% DC-200 on Chromasorb NAW 80-100 mesh

Temperature:
- Column = 90°C
- Detector = 250°C
- Injection Port = 150°C

Carrier:

Nitrogen	30 ml/min	Matheson Gas Co.
Hydrogen	14 psig	Matheson Gas Co.
Air	24 psig	

Range: 64 x 10

Sample Size: 0.6 ul of top layer

Notes on Method

This method is essentially the same as published by Cropper and Puttnams (J.S.C.C. 21,533,1970) for the determination of total fluoride in dental creams. It is based on an original publication of Bock and Semmler (Anal.Chem. 230,161,1967) and involves the following reaction:

$$R_3\text{-}SiCl + H_2O \longrightarrow R_3\text{-}SiOH + HCl$$

$$R_3\text{-}SiOH + H^+ + F^- \longrightarrow R_3\text{-}SiF + H_2O$$

The alkylchlorosilane is converted by water into the corresponding silanol. The silanol reacts selectively with fluoride to form fluorosilane, which can be extracted from the acidified solution quantitatively by means of G.C. as described by Fresen et al. (Pharm.Weekblad 103,909,1968)

Procedure

Preparation of Solution (A)

Weigh 0.4422 gm of NaF. Transfer to a 1000 ml volumetric flask and add deionized water up to 1000 ml (0.001 gm Fluorine/5 ml)

Preparation of Solution (B)

Weigh 0.1500 gm of n-Pentane solution. Transfer to a 250 ml volumetric flask and add toluene to 250 ml (0.00300 gm/5 ml)

Preparation of Solution (C)

Pipette 5 ml of fluoride solution (A) into a screw-cap tube, add 8 ml of conc. HCl and 5 ml of deionized water.
Cap and mix. Add 2 ml of trimethylchlorosilane, cap and mix.
Let stand for 15 minutes. Pipette in 5 ml of n-Pentane solution (B), mix and inject 0.6 ul of top layer.

Determination of Total Fluorine in Dentifrice

Weigh accurately 1.0 gm $\pm$ 0.1 gm of dentifrice into a screw-cap test tube. Add several glass beads and 8 ml of conc. HCl in parts.
Cap and shake, pausing several times to release pressure.
Add 8 ml of deionized water, cap and mix. Immerse in a boiling water bath for about 1 minute. Mix and let stand for 2 minutes, then cool under running water.

Add 1 ml of trimethylchlorosilane, mix and let stand for 15 minutes. Pipette in 5 ml of n-Pentane solution (B), mix and inject 0.6 ul of upper layer.

Calculation:

In the Solution (C)

Factor =

$$\frac{\text{peak height of fluorine (TMFS)}}{\text{peak height of n-Pentane}} \times \frac{\text{gm of n-Pentane in 5 ml of Sol.(B)}}{\text{gm of fluorine in 5 ml of Sol. (A)}}$$

In the dentifrice sample

gm of fluorine =

$$\frac{\text{peak height of fluorine (TMFS)}}{\text{peak height of n-Pentane}} \times \frac{\text{gm of n-Pentane in 5 ml of Sol.(B)}}{\text{Factor}}$$

$$\% \text{ of fluorine} = \frac{\text{gm of fluorine}}{\text{weight of sample}} \times 100$$

PPM of Fluorine = % Fluorine X 10,000

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #7

DETERMINATION OF TOTAL FLUORINE IN SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #7

DETERMINATION OF TOTAL FLUORINE IN SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

The objective of this method is to measure the total fluorine found in a sodium monofluorophosphate chalk dentifrice. This method is employed when there is interference from silica present in the dentifrice.

Reagents	Suggested Type or Source
n-Pentane 99%	Tridom Chemical Co. or equivalent
Toluene 99%	MCB Chromatoquality
HCl Conc.	Baker Analyzed Reagent
Trimethylchlorosilane 99%	Tridom Chemical or equivalent
NaF	Baker Analyzed Reagent
Test Tubes (screw-cap)	S.G.A.
Pipettes	S.G.A.
Beakers	S.G.A.
Methyl Ethyl Ketone	Baker Analyzed Reagent

Apparatus	Suggested Type or Source
Gas Chromatograph Model 5710A with dual flame ionization detector	Hewlett-Packard

Column: 6 ft. 1/8" OD stainless steel packed with 25% DC-200 on Chromasorb NAW 80-100 mesh

Temperature:
- Column = 90°C
- Detector = 250°C
- Injection port = 150°C

Carrier:

Nitrogen	30 ml/min	Matheson Gas Inc.
Hydrogen	14 psig	Matheson Gas Inc.
Air	24 psig	

Range: 64 x 10

Sample Size: 0.6 ul of top layer

Notes on Method

This method is essentially the same as published by Cropper and Puttnams (J.S.C.C. 21,533,1970) for the determination of total fluoride in dental creams. It is based on an original publication of Bock and Semmler (Anal.Chem. 230,161,1967) and involves the following reactions:

$$R_3\text{-}SiCl + H_2O \longrightarrow R_3\text{-}SiOH + HCL$$

$$R_3\text{-}SiOH + H^+ + F^- \longrightarrow R_3\text{-}SiF + H_2O$$

The alkyl-chlorosilane is converted by water into the corresponding silanol. The silanol reacts selectively with fluoride to form fluorosilane, which can be extracted from the acidified solution quantitatively by means of G.C. as described by Fresen et al. (Pharm. Weekblad 103,909,1968)

Procedure

Preparation of Solution (A)

Weigh 0.4422 gm of NaF and carefully transfer to a 1000 ml volumetric flask; then fill to volume with deionized water (0.001 gm fluorine/5ml).

Preparation of Solution (B)

Weigh 0.1500 gm of n-Pentane. Transfer to a 250 ml volumetric flask, and add toluene to 250 ml (0.003 gm/5 ml).

Preparation of Solution (C)

Pipette 5 ml of fluoride solution (A) into a screw-cap tube, add 5 ml of deionized water and 8 ml of conc. HCl, cap and mix. Add 2 ml of trimethylchlorosilane, cap and mix; let stand for 15 minutes. Pipette in 5 ml of n-Pentane solution (B). Cap and mix. Inject 0.6 ul of top layer.

Determination of Total Fluorine in Dentifrice

Accurately weigh 2.0 gm of the dentifrice into a screw-cap tube. Add 2.5 ml of conc. HCl and a few glass beads. Cap and shake, pausing several times to release pressure. Add 10 ml of deionized water, cap and mix. Release the pressure, cap tightly and immerse in a boiling water bath for 1 minute. Remove and let stand for about 2 minutes, then cool under running cold water.

Add 1 ml of trimethylchlorsilane, cap and mix. Let stand for 15 minutes then add 5 ml of n-Pentane solution (B) and 3 ml of methyl ethyl ketone, cap and mix. Let settle for about 5 minutes and inject 0.6 ul from top layer.

Calculation

In the Solution (C)

Factor =

$$\frac{\text{peak height of Fluorine (TMFS)}}{\text{peak height of n-Pentane}} \times \frac{\text{gm of n-Pentane in 5 ml of Sol.(B)}}{\text{gm of Fluorine in 5 ml of Sol.(A)}}$$

In the dentifrice sample

gm of Fluorine =

$$\frac{\text{peak height of Fluorine (TMFS)}}{\text{peak height of n-Pentane}} \times \frac{\text{gm of n-Pentane in 5 ml of Sol.(B)}}{\text{Factor}}$$

$$\% \text{ of Fluorine} = \frac{\text{gm of Fluorine}}{\text{weight of dentifrice}} \times 100$$

PPM of Fluorine = % Fluorine X 10,000

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #8

DETERMINATION OF TOTAL FLUORINE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #8

DETERMINATION OF TOTAL FLUORINE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

The objective of this method is to measure the total fluorine found in sodium monofluorophosphate chalk dentifrice.

Reagents	Suggested Type or Source
HCl Conc.	Baker Analytical Reagent
Amberlite IR 120 H	Rohm & Haas
Sodium Acetate	MCB Inc.
Polyethylene Beakers	S.G.A.
Pipettes	S.G.A.
Volumetric Flasks	100 ml - 250 ml
NaF	Baker Analytical Reagent
Deionized Water	

Apparatus	Suggested Type or Source
Ionalizer Specific Ion Meter, Model 407 A	Orion Research Corp.

Procedure

Weigh accurately 6.0 gm $\pm$ 0.1 gm (w) of dentifrice into a polyethylene beaker, slurry with about 5 ml of deionized water and carefully add concentrated hydrochloric acid in small portions until effervescence ceases. Add an additional 2 ml of concentrated hydrochloric acid, cover the beaker and set aside for 45 minutes at room temperature.

Transfer the solution quantitatively to a 100 ml volumetric flask and bring to volume with deionized water.

Slurry sufficient resin (approx. 25 gm) in deionized water to make a column 20-23 cm. long. Pipette 20 ml of the sample solution into the column and elute with deionized water at a rate of about 2 drops per second.

Collect the eluate in a 250 ml graduated flask, adjust the volume to 250 ml with sodium acetate solution (15% w/v) and mix thoroughly.

Standardize the specific ion meter by setting the scale at 1 ppm fluorine and the upper end of the scale at 10 ppm fluorine using the two sodium fluoride solutions.

Determine the meter reading (A) of the sample in sodium acetate, immersing the specific electrode in about 40 ml of the solution contained in a polyethylene beaker, making due allowance for the electrode response time.

Calculation

$$\text{Total Fluorine (ppm)} = \frac{A \times 12,500}{W}$$

Where A = ppm Fluorine from meter reading
W = weight of paste taken in grams

9

TEST METHOD #9

Title: Determination of Soluble Fluoride in Stannous Fluoride - Calcium Pyrophosphate Toothpaste Supernatants by Fluoride Electrode

Recommended for the Following Systems:

a. Stannous Fluoride - calcium pyrophosphate

b. Stannous Fluoride - insoluble sodium metaphosphate

Test Method #9

Determination of Soluble Fluoride in Stannous Fluoride-Calcium Pyrophosphate Toothpaste Supernatants by Fluoride Electrode

Scope

This method is applicable for the determination of soluble, and therefore available, fluoride in stannous fluoride-calcium pyrophosphate toothpastes under normal brushing times and dilutions. The time of slurrying and the time until centrifuging begins are critical and must be rigidly adhered to if reproducibility is to be attained. The emf developed by a calibrated Orion fluoride electrode in contact with the toothpaste supernatant is related to the ppm F^- in the supernatant. Stannous fluoride toothpastes supernatants require the addition of an ammonia buffer-EDTA reagent. A calibration curve is also required and is included in this method.

Reagents

Ammonium Hydroxide	A.C.S. grade
Sodium Nitrate	A.C.S. grade
Sodium Fluoride	A.C.S. grade
Ammonium Chloride	A.C.S. grade
(Ethylenedinitrilo) tetraacetic Acid, Disodium Salt ($Na_2EDTA \cdot 2H_2O$)	A.C.S. grade
Deionized Distilled Water	Laboratory supply, oxygen-free

Apparatus

Pipettes	5, 25, 50 ml.
Magnetic Stirrer	Labline "Magne-stir" or equivalent
Stirring Bars	Teflon coated, 7/8 inch, or equivalent.
Polyethylene Bottles	250, 500 ml.
Volumetric Flasks	50, 250 ml.
Pasteur Pipette	
Expanded Scale pH meter	Beckman Expandomatic or equivalent.
Fluoride Specific Ion Electrode	Orion Research, Inc., 11 Blackstone St., Cambridge, MA 02139; Model 94-09-00 (Available from Fisher Scientific Products, etc.)

OR

Combination Fluoride & Reference Electrode Model No. 96-09 (Orion)

NOTE: Two electrodes should be on hand if heavy use is anticipated.

Balance	Harvard Trip or equivalent
Single Junction Reference Electrode	Orion Research, Inc., Model No. 90-01
Electrode Holder	Orion Research, Inc., Model No. 92-00-01

Centrifuge, high speed	International Model CS, UV, 2N, or 2-EXD with high-speed attachment.
Centrifuge Tubes	25 ml, International No. 298
Plastic Beakers	100 ml disposable
pH paper	Hydrion, or equivalent, with 0.2 pH interval or less.
Stirring Rods	8-inch, with about 2-inch piece of Tygon tubing slipped over one end, such that an inch of Tygon extends beyond the glass.
Graph paper	Codex Brand No. 31,286 (two-cycle semi-log graph paper)

Preparation of Oxygen-free distilled water

Distilled water is placed in an Erlenmeyer flask or gallon jug and a nitrogen line, ending in a gas dispersing tube, is inserted into the water. Vigorously bubbling nitrogen through the water for a minimum of 30 minutes will render the water sufficiently oxygen-free for this analysis.

Sodium Fluoride Stock Solution (0.500M)

Dry about 10 g sodium fluoride at 105°C for 3 hours. Store the sodium fluoride in a desiccator and allow to cool. Weigh out 5.249 g of the dried reagent, dissolve in distilled deionized water, dilute to 250 ml in a volumetric flask. Minimize the time of contact with glass. Store in a 250 ml polyethylene bottle.

Sodium Nitrate Stock Solution (1.00M)

Weigh out 21.25 g of sodium nitrate, dissolve in distilled deionized water, dilute to 250 ml in a volumetric flask.

Preparation of Sodium Fluoride-Sodium Nitrate Calibration Solution (0.005 M NaF, 0.1 M $NaNO_3$)

Pipette 5 ml of the sodium fluoride stock solution and 50 ml of the sodium nitrate stock solution into a 500 ml volumetric flask. Dilute to the volume with distilled deionized water. Store in polyethylene bottle.

Stock Ammonia Buffer Solution

Place 67.5 g ammonium chloride in a one-liter flask and dissolve with about 250 ml of distilled deionized water. Add 570 ml of concentrated ammonium hydroxide to the flask and dilute to volume with distilled deionized water. Store in a polyethylene bottle. Discard solution within seven days after preparation. Use of solution older than seven days may result in erroneous readings.

Preparation of Dilute Ammonia Buffer-EDTA Reagent Solution (0.1 M EDTA)

Place 37.2 g of (ethylenedinitrilo) tetraacetic acid disodium salt (EDTA) in a one-liter volumetric flask. Add 250 ml of the stock ammonia buffer to the flask and swirl to dissolve the EDTA. Dilute to volume with distilled deionized water. Store in a polyethylene bottle.

Potential Sclae of pH Meter

The Beckman Expandomatic pH meter should be used in the expanded sclae and -MV modes. The potential range necessary for fluoride activity measurements is 0 to -200 MV. A standard solution of 0.005M NaF, 0.1M $NaNO_3$ will be arbitrarily assigned a reading of -100 MV; that is, the meter should be adjusted to read -100 MV (the center of the MV scale) when the fluoride and reference are contacting the calibrating solution. Before the electrodes are removed from a solution the "STANDBY" button on the meter is pressed.

Calibration of Orion Fluoride Specific Ion Electrode

The instruction book for the electrode should be read in order that the analyst may become familiar with proper electrode handling and optimum operating conditions. The fluoride electrode must be calibrated every two weeks to establish the potential response versus concentration of free fluoride. The Orion single junction reference electrode should be used as the reference electrode. The following three solutions are necessary and sufficient for calibrating the electrode:

1. 0.05 M NaF, 0.1 M $NaNO_3$
2. 0.005 M NaF, 0.1 M $NaNO_3$
3. 0.0005 M NaF, 0.1 M $NaNO_3$

They can be prepared from stock solutions. Solution 2 (0.005 M NaF, 0.1 M $NaNO_3$) should be read first and the potential adjusted to read - 100.0 MV (estimated to the nearest 0.5 MV). The solutions should then be read in the following order 2, 1, 2, 3, 2; the potential of 2 should not drift more than $\pm$ 1 MV and should be readjusted to -100.0 MV in any case.

The solutions should be stirred but not so vigorously as to allow bubbles to form and contact the fluoride electrode membrane crystal. At least one minute should be allowed for the electrode to reach a steady reading. A plot of MV versus log concentration on semi-log paper should yield a slope of 59$\pm$ 1 MV. (That is, the potential difference between solutions 1 and 2, and 2 and 3 should be 59$\pm$ 1 MV). If the electrode does not behave in this manner, it should be exchanged for a new electrode under the warranty terms with Orion Research, Inc.

Preparation of Supernatants

Two samples can conveniently be prepared at the same time. Discard the first inch of toothpaste from the tube, then weigh 10.0 grams of each paste into 100 ml beakers. Pipette 30 ml of oxygen-free distilled water into each of the beakers and then set a clock-timer for 5 minutes before starting the slurrying. Start the timer and then, using a separate Tygon-tipped stirring rod in each beaker, alternately slurry each sample (10-15 seconds each) until 2 minutes have elapsed. Pour 22-25 ml of the uniform slurries into centrifuge cups, taking care to balance the two cups very precisely. Five minutes after starting to slurry the pastes (by the clock-timer), start the centrifuge and spin the slurries for 30 minutes at 11,000 r.p.m. Two other pastes may be started at this time, and the centrifuge may be stopped to add these two samples at the end of their 5-minute time period. All four samples are then centrifuged for 30 minutes.

Operation

Pipette 15 ml of the toothpaste supernatant into a 100 ml plastic beaker. Pipette 10 ml of the dilute ammonia buffer-EDTA reagent solution into the same beaker. Before measuring the fluoride activity of the supernatant, the electrode should be calibrated to read -100 MV when measuring the fluoride activity of the

calibrating solution, 0.005 M NaF, 0.1 M $NaNO_3$. The fluoride and reference electrodes are then lowered into the test solution and the -MV button pressed. Take the potential reading after one minute of stirring to allow the electrode to reach a steady value. The potential should be read to the nearest 0.5 MV and that value recorded. The electrodes should then be thoroughly washed with distilled water, especially the membrane crystal of the fluoride electrode. The electrodes are then gently wiped dry with absorbent tissue. The calibrating solution is then remeasured. If the potential is more than $\pm$ 1 MV different from -100 MV, it should be adjusted to -100 MV and the potential of the supernatant remeasured.

Determination of Fluoride Concentration in Supernatants as ppm

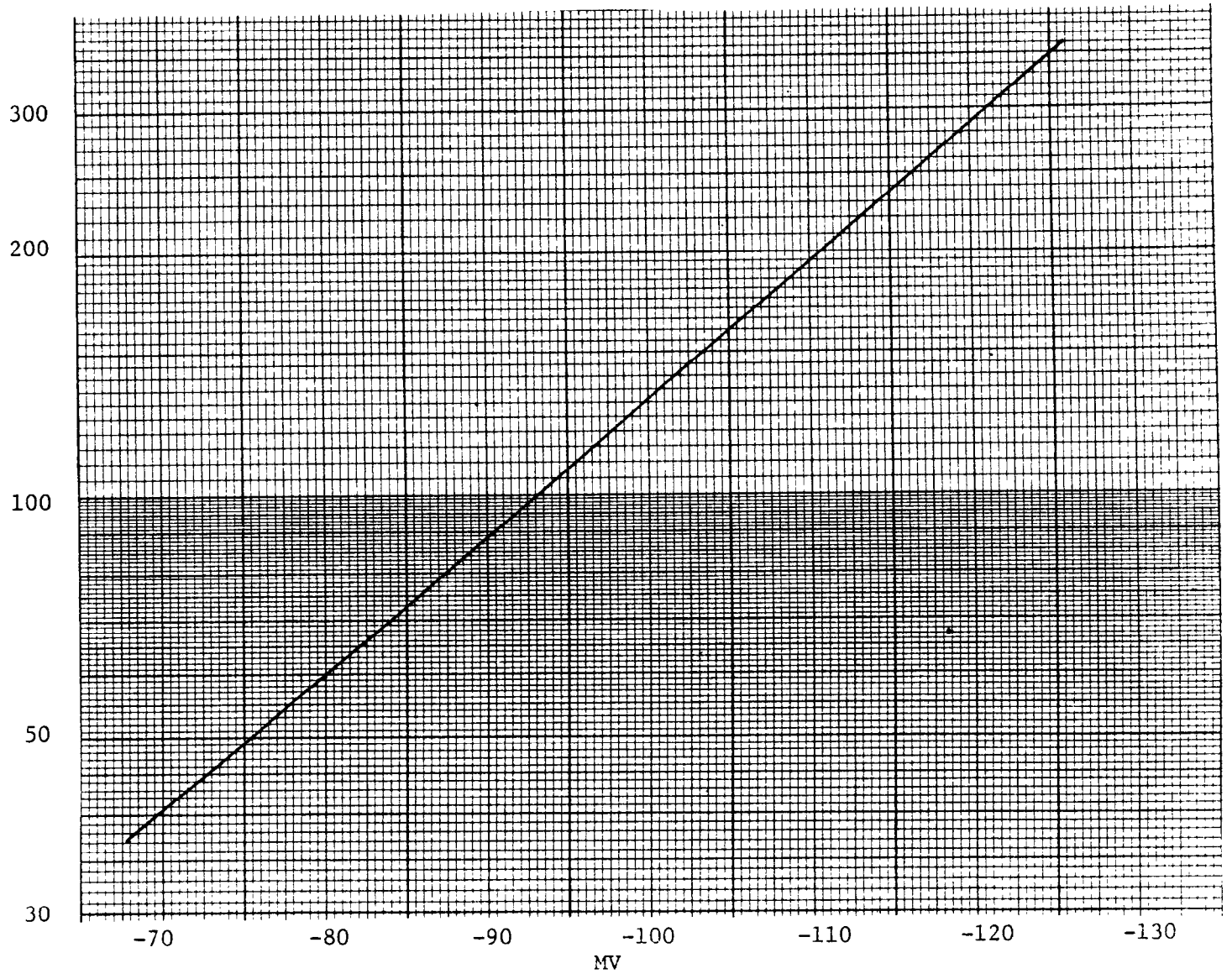
A calibration curve similar to the one shown in this method should be used. The "ppm F" is the vertical or log-scale and "MV" is the horizontal axis. When the calibrating solution is adjusted to read -100 MV, the measured potential of the test solution is found on the curve, and the ppm F^- in the original supernatant is read and recorded. This calibration curve has a dilution factor of 15/25 incorporated into it, so no correction is necessary. The calibration curve may be prepared by plotting the following data on two-cycle semi-log graph paper and drawing a straight line through the points.

Log Scale ppm F^-	Linear Scale MV
60	-80.0
130	-99.7
280	-119.5

Temperature Control of the Test Solutions

In order to prevent the test solutions from being heated by the magnetic stirrer, insulate the test solution. Two suggested methods are (1) place a section of cardboard on the stirrer or (2) use a triple cup thickness. The potential of the calibrating solution and each test solution should be measured at room temperature (70-80°F).

Calibration Curve for Soluble Fluoride
in Stannous Fluoride - Calcium Pyrophosphate Toothpaste



10

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #10

DETERMINATION OF SOLUBLE FLUORIDE (IONIC)

Recommended for the following systems:

a. Stannous fluoride and silica abrasive.

TEST METHOD #10

DETERMINATION OF SOLUBLE FLUORIDE (IONIC)

Principle

The sample is a centrifuged aqueous extract of toothpaste.

The fluoride content of this solution is determined by the potentiometric technique of multiple standard addition. In this technique incremental volumes of a standard fluoride solution are added to the sample solution. The potentials developed at a fluoride sensitive electrode vs. a suitable reference electrode are observed at each step. These stepwise changes in potential, measured in millivolts, are related to the original fluoride concentration of the sample solution by a computation derived from Nernst's equation (*).

Equation (1)

$$C_x = \frac{C_S}{10^{\frac{\Delta E}{slope}}\left[1 + \frac{V_x}{V_s}\right] - \frac{V_x}{V_s}}$$

Term C_x is the unknown concentration, C_S is the fluoride concentration in the standard solution, ΔE is the potential change caused by the addition of the known increment of fluoride solution, "slope" is the slope of the Nernst equation, V_S is the volume of fluoride standard added, and V_x is the original volume of sample solution.

Apparatus

Centrifuge, Servall Type SP: Ivan Sorvall, Inc., Norwalk, Conn.
Centrifuge tubes, round bottom, polypropylene, 50-ml, such as SGA Scientific Inc. Item No. C-3512-2.
Centrifuge tube closures to fit 50-ml centrifuge tubes, such as SGA Scientific Inc. Item No. C-3512-4.
Corning Digital 112 Research pH/mV Meter.
Orion Fluoride Specific-Ion-Sensitive Electrode 94-09A.
Orion Single-Junction Reference Electrode 90-01 and a supply of Orion Reference Electrode Filling Solution 90-00-01. DO NOT USE ANY OTHER FILLING SOLUTION!
Magnetic Stirring Apparatus.
Erlenmeyer flask, 50-ml.
Burets, 10-, 25- and 50-ml, meeting Class A specifications.
Graduated cylinders, 50-ml.
Volumetric Flasks, 250-ml and 1000-ml sizes.
Pipets, 10-ml, 20-ml, 25-ml sizes, Class A.
Heath EUA-20-12 pH/mV Test Box.

*Karlberg, B. Anal. Chem. 43, 1911-1913 (1971).

Reagents

Sodium Hydroxide Pellets, ACS Reagent Grade. Prepare a liter of Sodium Hydroxide Solution, 10% w/v in distilled water. Store in a polyethylene bottle.

Glacial Acetic Acid, ACS Reagent Grade. Prepare 2 liters of Acetic Acid Solution, 10% v/v in distilled water.

Phenolphthalein Indicator Solution, prepare as in A.M. 0.020 for laboratory use.

Sodium Fluoride, ACS Reagent Grade, preferably Fisher Certified S-299. Prepare the solution shown below.

Master Fluoride Solution (2500 ppm w/v Fluoride, pH 5).
Dissolve 5.526 grams of reagent grade ACS-specification sodium fluoride in water, add 40 ml of 10% w/v sodium hydroxide solution, then add 80 ml of 10% v/v acetic acid solution and mix. Transfer the mixture quantitatively to a 1000-ml volumetric flask. Make the contents of the flask to the mark with water near 25°C and mix thoroughly.
Transfer the solution to a polyethylene bottle for storage.

Procedure

A few instrumental preliminaries are needed. The fluoride electrode is preferably stored dry, covered with a cap to protect the fragile crystal membrane. The reference electrode is also stored with a protective cap to prevent evaporation. Remove these caps, inspect both electrodes for obvious mechanical damage, and if none is evident immerse the pair in stirred distilled water. Refill the reference electrode if necessary, using only the Orion filling solution.

The Corning meter may be left on permanently. If there is any suspicion that the meter is not functioning properly, use the Heath test box to check the meter, remembering that the Corning meter is more accurate than the test source, and this procedure will only detect gross error.

Preheat a hot plate while samples are being extracted. An 8" Chromalox 1500-watt hot plate is recommended for fastest warmup time.

First, prepare the "available" fluoride extract. Weigh 3.5 grams of toothpaste, to an accuracy of 1 mg, into a centrifuge tube.

Keep the centrifuge tube capped as much as possible during the entire extraction procedure.

Add from the 50 ml buret 1- to 2-ml increments of oxygen-free water, thoroughly mixing with the paste (use a glass rod) after each addition until a smooth slurry is obtained (about 10 ml of water). Continue the addition of water until the total volume added is 10 times the weight of paste taken.

Mix by stirring, rinse off the glass rod with 1 or 2 ml of oxygen-free water from the buret, cap the tube, and centrifuge at 5000 rpm for 15 minutes. Read the total volume of water added from the buret to the nearest 0.1 ml.

Immediately decant the supernatant liquid into a 50-ml Erlenmeyer flask. Aliquots of this solution are used for "available" fluoride determinations.

The fluoride in the extract is then determined.

Tare a 300-ml Pyrex beaker. Accurately weigh into it by difference 20-25 grams of "available fluoride" extract. Add 10 ml of 10% w/v sodium hydroxide solution from a pipet. Add 50 ml of water from a graduate.

The next operation is slightly hazardous, and appropriate eye protection is demanded. Beaker tongs and gloves must also be used. Using a 10-inch glass stirring rod for constant stirring to avoid violent bumping, heat the contents of the beaker to a rolling boil for approximately a one-minute boiling period. Vigorous foaming will occur, and the mixture will become almost clear. Without interrupting the stirring, lift the beaker from the hot plate until the foaming subsides. Stop stirring when foaming stops, then let the mixture cool for approximately ten minutes.

Quantitatively transfer the mixture from the beaker to a 250-ml volumetric flask without using excessively large amounts of water. A small quantity (several drops) of phenolphthalein indicator solution may be added to the flask if desired. Pipet 20 ml of 10% v/v acetic acid solution into the flask, swirling it gently as the addition is made. Neutralization will occur before the entire quantity of acetic acid solution has been added. Make the contents of the flask to the mark with water and mix. Invert the beaker on a towel for a few minutes to drain.

Without using any additional water, transfer the contents of the flask back into the original beaker, draining the flask well by use of a small ring clamp. A small transfer error is involved here, but its size is trivial. Clean the volumetric flask before the film of solution on its surface has dried, or it will be difficult to clean. Cover the beaker with a watch glass or Parafilm until the determination can be completed. Slow precipitation of hydrous silica may occur. Wait at least ½ hour to allow nucleation of hydrous silica to begin. The next operation may be performed immediately or within 24 hours irrespective of the sample appearance. Overnight standing ordinarily results in copious precipitation of hydrous silica, but this has no noticeable effect on the results.

The multiple additions procedure requires several precautions. The sample temperature must be within a few degrees of room temperature, and most stirrers require thermal insulation to keep the sample from heating. A square of plastic foam covering the stirrer top is advised. The electrode immersion depth should be fixed, and the stirring rate should be fixed but not vigorous enough to create a vortex. The electrodes should be wiped gently with a tissue before every immersion. If a blank is to be analyzed because of suspected fluoride contamination, the blank must be the first analysis of the day, because the electrode has a definite "memory". Analysis of blanks is not necessary except when reagent lots are changed.

Record the mV reading of the electrode pair in distilled water. Immerse the electrodes in the stirred sample solution, and follow the timing schedule below. Precise timing is not required, but these minimum times must be observed.

(1) Record the initial potential after 5 minutes. (Exception: Wait ten minutes if this is the first sample of the day).

(2) Add 1.00 ml of 2500 ppm fluoride master solution from a buret, and record the potential 2 minutes after the addition is complete.

(3) Four additional 1.00-ml volumes of 2500 ppm fluoride master solution are added next, so for buret readings of 2.00, 3.00, 4.00, and 5.00 record the potential 1 minute after the addition is complete.

(4) After each sample, rinse the electrodes briefly with distilled water, then return them to stirred distilled water until the reading is approximately 120 mV higher than the initial reading from a fluoride-containing toothpaste. Record these readings also. A properly functioning fluoride electrode should attain these responses in 2 minutes or less. It is not necessary or desirable to wash the electrode exhaustively with water between analyses, because of the "memory" effect.

Computations

Fluoride is calculated in accordance with Equation (1), above. Five values of this equation are computed for each of five additions of a standard fluoride solution. The mean of these and its estimated standard deviation are calculated, too.

C_x is the unknown fluoride concentration of the sample solution.

C_s is the fluoride concentration of the standard solution.

ΔE_1 is the incremental potential change in millivolts observed upon addition of the first volume of standard fluoride.

V_x is the original volume of the sample solution in milliliters.

V_s is the totally added volume of the standard fluoride solution in milliliters.

A value of -59.16 millivolts per decade change of fluoride activity may be assumed. It is convenient to express fluoride concentrations in micrograms F per milliliter. Results are then recomputed to the paste basis.

(The equation is not as formidable as it appears to be. If a fluoride electrode has a negative logarithmic response to fluoride activity, it is intuitively obvious that fluoride activity will be an inverse antilogarithmic function of electrode potential. The remaining terms in the equation are volume corrections).

A volume-corrected Gran Function (2) is also calculated for each of the five additions. This antilogarithmic function provides additional confidence in the performance of the method.

The true slope and standard potential of the electrode system can be approximated from a calibration line by nonlinear regression analysis of calibration data of the form $y = a + b \log_{10} x$. However, these are best determined by the calculation of Brand and Rechnitz (3), which also provides an independent estimate of fluoride concentration. This computation is a formidable numerical analysis routine demanding the facilities of a computer. The earlier computations are readily programmable.

Example

Assume a 2.500 gram sample of dentifrice containing its target level of 1000 µg F per gram of paste, or a total of 2500 µg F. This 2500 µg quantity of F dissolved in 250 ml total volume gives a fluoride concentration of 10.000 micrograms per milliliter. Now add five separate 1.00-ml increments of a standard fluoride solution with 2500 µg F/ml. Observe the response of a fluoride electrode with perfect slope (-59.16mV) and a standard potential of -180 mV at 1 molar F activity. The following conditions should prevail:

Ml added	µg F present	Volume, ml	ppm F	pF Molarity	E in Millivolts
None	2500	250	10.00	3.27875	13.9711
1.00	5000	251	19.9203	2.97946	-3.7353
2.00	7500	252	29.7619	2.80509	-14.0507
3.00	10,000	253	39.5257	2.68187	-21.3403
4.00	12,500	254	49.2126	2,58668	-26.9722
5.00	15,000	255	58.8235	2.5092	-31.5556

Ml added	Delta EqmV	Volume-Corrected Gran Function	Computed F µg/ml of Original Solution by Equation (1)
None	(Initial)	-	-
1.00	-17.71	2.0003	9.9972
2.00	-28.02	2.9998	10.001
3.00	-35.31	3.9998	10.0007
4.00	-40.94	4.9994	10.0016
5.00	-45.53	6.0008	9.9984

The last column obiously confirms the target level of 1000 µg F per gram on the original sample of dentifrice in this example. Available fluoride calculations are similar.

(1) Karlberg, B. Anal. Chem. 43 1911-1913 (1971).
(2) Gran, G. Analyst 77 661-671 (1952)
(3) Brand, M.J.D., and Rechnitz, G.A. Anal. Chem. 42 1172-1177 (1970).

11

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #11

TITLE: DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #11

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

PRINCIPLE

The method for total soluble fluoride is based on the same principle as for total fluoride, except that an aqueous extract is used instead of an acid extract.

APPARATUS REQUIRED

Same as for the total fluoride method.

REAGENTS REQUIRED

Same as for the total fluoride method except for the absence of perchloric acid.

PROCEDURE

1. Accurately weigh (to the nearest milligram) 20 grams of sample into a 100 or 150 ml beaker.
2. Add 60 ml of water. Slurry to disperse the sample and break up any lumps.
3. Quantitatively transfer to a 100 ml volumetric flask and dilute to volume with water.
4. Mix vigorously using a magnetic stirring bar and stirrer for at least 30 minutes.
5. Centrifuge a portion of the above slurry until clear.
6. Pipette 15.0 ml of the clear supernatant liquid into a 500 ml volumetric flash and dilute to volume with water. Mix well.
7. Transfer a portion to the plastic sample cups. See Note A.
8. With the fluoride manifold on the proportioning pump and all the lines in water lower the roller head to start the instrument. Start the vacuum pump and adjust to 5 or 6 inches of Mercury. Keep the vacuum constant once the test is started.

9. Run water through the lines for a few minutes to check the flow. Dip all lines in their respective solutions.

10. Set the sampler table with the 20, 1-2 samples per hour cam and load the tray with 3 - 3.5 ml plastic cups.

11. The cups are filled alternately with standards and samples. Since each laboratory's needs will vary, no fixed procedure for this step can be made. It is recommended that the first 22 cups, which will approximate a one hour run, be filled as follows: See Note B.

Cups	1 - 4	1.12	Standard (mg F/200 ml)
Cups	5 - 10		Sample Solutions*
Cups	11 - 13	1.44	Standard (mg F/200 ml)
Cups	14 - 19		Sample Solutions*
Cups	20 - 22	1.28	Standard (mg F/200 ml)

* Two cups are filled for each sample and the average of the two analyses used in the calculation. Single analyses may be run once the instrument has been shown to yield consistent results.

12. The succeeding cups are filled alternately with samples (in duplicate), three 1.44 standards, samples (in duplicate), and three 1.12 standards.

13. Turn on the colorimeter and recorder.

14. When the baseline is established, set the absorbance at 0.01 and start sampling, making certain that the sampling probe dips well into the cups. It is essential that a steady bubble pattern of small bubbles be established after the working reagent is added. A steady baseline will be assurance of this.

15. After the last sample has been aspirated, turn off the sampler and discard the cups.

16. After the last sample trace appears on the recorder chart, dip all the lines in water and flush the system.

17. After the instrument lines are washed for about 15 minutes, lift up the roller head, release the manifold and stop the vacuum pump.

18. Remove the recorder chart and record the absorbance values of each peak at the peak height. Ignore the first peak from the 1.12 standard.

CALCULATIONS

The calculations are performed on sets representing each hour of running time (Steps 11 and 12).

1. Calculate the average absorbance of each standard by adding the absorbance values and dividing by three. The first cup peak must be ignored.

2. For each standard calculate the K value as follows:

$$K = \frac{\text{Milligrams F per 200 ml}}{\text{Average Absorbance of each Standard}}$$

3. Calculate the average K_{AV} value for each hour run by adding the standard K values and dividing by the number of standards represented. It is important to determine the K_{AV} for each hour of operation and to use the value obtained for calculation of results for samples included in that run. See Note C.

4. Calculate the Total Fluoride as follows:

$$\frac{(K_{AV} \times A)}{S} \times 100 \times 0.06 = \text{Total Soluble Fluoride as F}$$

Where K_{AV} = Average K value (calculation 3)
A = Average absorption of samples
S = Sample weight in milligrams (Step 1).

NOTES:

A. The placebo fluoride standards used for soluble fluoride are the same as those used in the total fluoride method. If the soluble fluoride is less than 1 mg/200 ml, then appropriate, lower standards should be used.

B. The indicated order in which the cups are filled is only given as an example and the samples and standards may be interchanged as desired.

C. It has been observed that the system may need to be standardized continuously. Thus, hourly averages of K for standardization are suggested. For instance, a run of 3 hours would require three average K values and each K value would be used in calculating the % fluoride content of the inclusive unknowns.

12

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #12

TITLE: DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #12

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

PRINCIPLE

Total Soluble Fluoride is based upon the removal of water insoluble ingredients by centrifugation. The supernatant liquid is in the presence of calcium oxide. The fluoride is distilled from a perchloric acid solution of the ashed residue and reacted in solution with Eriochrome Cyanine R and Zirconyl Chloride. The bleaching effect of the fluoride on the Zirconium lake is measured spectrophotometrically and the fluoride level is obtained by reference to a standard graph.

METHOD

APPARATUS REQUIRED

1. Steam Distillation Apparatus of borosilicate glass with band heater as shown in Figure 1, LaPine Scientific Co., Irvington-On-Hudson, N. Y.

2. Glas-Col Heating Mantle, hemispherical to fit 2000 ml flask.

3. Variable Transformers (2) such as the "Powerstat" Type 116, Superior Electric Co., Bristol, Conn., or "Variac", Type V-5, General Radio Co., Cambridge, Mass. These transformers are available from most laboratory supply houses.

4. Centrifuge, capable of speeds up to 10,000 rpm.

5. Centrifuge tubes, Nickel, 1" x 4", (2).

6. Mechanical stirrer.

7. Nickel dish - 30 ml.

8. Muffle furnace.

REAGENTS REQUIRED

Zirconyl Chloride Solution ($ZrOCl_2$)

Dissolve 0.265 gram of Zirconyl Chloride Octahydrate ($ZrOCl_2 \cdot 8H_2O$) in 50 ml of water. Add 700 ml of conc. Hydrochloric Acid and cautiously dilute to volume with water at room temperature in a 1000 ml volumetric flask. Mix well.

NOTE:

0.220 gram of Zirconyl Nitrate Dihydrate ($ZrO(NO_3)_2 \cdot 2H_2O$) may be used in place of Zirconyl Chloride Octahydrate.

Silver Perchlorate Solution ($AgClO_4$) 50%

Dissolve 250 grams of anhydrous Silver Perchlorate ($AgClO_4$) in 400 ml of water and dilute to 500 ml.

Eriochrome Cyanine R Solution

Dissolve 1.8 grams of Eriochrome Cyanine R (obtainable from Geigy Dyestuffs Div., Geigy Chemical Corp., N. Y., N. Y.) in 100 ml of water and dilute to 1000 ml.

Sodium Hydroxide Solution (NaOH), 5%

Dissolve 5 grams of Sodium Hydroxide in 50 ml of water and dilute to 100 ml.

Calcium Oxide (CaO): The grade obtainable from Fisher Scientific Co., Catalog No. C-117 (0.005% Fluoride) is recommended.

Sodium Fluoride (NaF) Reagent Grade.

Perchloric Acid ($HCLO_4$), 70%.

Hydrochloric Acid Solution (70 ml conc. HCl + 30 ml water) - Baker or Mallinckrodt HCl is recommended.

PREPARATION OF STANDARD CURVE

1. Accurately weigh 0.8842 gram of Sodium Fluoride and transfer to a 1000 ml volumetric flask. Add 500 ml of water and shake to dissolve the sample. Dilute to volume with water at room temperature and mix well. Store in a polyethylene bottle.

2. Pipet 10 ml of the sample solution from step 1 into a 1000 ml volumetric flask. Dilute to volume with water at room temperature and mix well. Each ml of this solution contains 0.00400 milligram (4 micrograms) of F. Store in a polyethylene bottle.

3. Using a buret, deliver 5.00 ml, 7.50 ml, 10.00 ml, 12.50 ml and 15.00 ml aliquots of the dilute solution from step 2 into separate 100 ml volumetric flasks. Add approximately 50 ml of water. Pipet 5 ml of Eriochrome Cyanine R solution and 5 ml of Zirconyl Chloride solution into each flask. Dilute to volume with water and mix well.

4. Using 1 cm cells in the Beckman DU Spectrophotometer, measure the absorbance of each solution from step 3 at 527.5 millimicrons. Use the reference solution as a blank. See step 14.

PROCEDURE

1. Accurately weigh 20 ± 0.2 grams (to the nearest 0.1 milligram) of sample into a 150 ml beaker. Add 60 ml of water and mix thoroughly with a stirring rod and then with a magnetic stirrer for 15 minutes.

2. Quantitatively transfer the dispersion to a 100 ml glass stoppered mixing cylinder using water to complete the transfer. Dilute to the 100 ml mark with water, stopper the cylinder and shake vigorously.

3. Transfer the dispersion from step 2 in equal amounts to 2 Nickel centrifuge tubes and centrifuge at 10,000 rpm for 20 minutes.

4. Pipet 10 ml of the clear supernatant liquid into a 30 ml Nickel dish. Add 0.5 grams of CaO and mix well.

5. Place the dish on a steam bath first to dry, then under a heating lamp and evaporate to dryness and char. Finally ash in a muffle furnace maintained at 560-600°C for 1/2 hour. At no time should the sample be permitted to ignite and burn with a flame.

6. Cool to room temperature and transfer the main contents of the dish with about 30 ml of water to the fluoride distilling flask containing approx. 0.5 gm of glass wool.

7. Add about 10 ml of 70% Perchloric acid to the dish. Mix, and with the aid of water, transfer to the distilling flask. Add an additional 40 ml of the 70% Perchloric acid and 1 ml of Silver Perchlorate to the sample solution in the flask. Place a 500 ml volumetric flask under the distillate outlet.

8. Turn on the transformer connected to the heating mantle surrounding the steam generator and set at the highest dial reading (about 130). Leave the Hoffman clamp open until the water boils.

9. Turn on the transformer connected to the band heater and set the dial at 100.

10. When the temperature in the distilling flask is approximately 130°C and the water is boiling in the steam generator, steam is admitted to the distilling flask by closing the Hoffman clamp. The temperature in the distilling flask is maintained at 130° ± 2°C by temporarily turning off the transformer connected to the band heater if the temperature reaches 137°C and turning it on again if the temperature drops to 135°C. The installation of an automatic temperature control, known as the "Therm-O-Watch," to the band heater and thermometer of the distillation unit will allow the operator to perform other duties while the distillation is in progress. The "Therm-O-Watch" unit (Model S-6) is obtainable from Instruments for Research and Industry, 108 Franklin Avenue, Cheltenham, Pa.

11. Collect about 450 ml of distillate. Turn the unit off by opening the Hoffman clamp and shutting off both transformers.

12. Neutralize the distillate to litmus by the dropwise addition of 5% Sodium Hydroxide solution and dilute to volume with water at room temperature. Mix well.

13. Pipet a 10 ml aliquot of the solution from step 12 into a 100 ml volumetric flask. Add approximately 50 ml of water. Using micro burets, measure 5 ml of Eriochrome Cyanine R solution and 5 ml of Zirconyl Chloride solution into the flask. Dilute to volume and mix well.

14. Using 1 cm cells in the Beckman DU Spectrophotometer, measure the absorbance of the solution at 527.5 millimicrons versus a reference solution prepared as described in step 15.

15. The reference solution is prepared as follows: Measure 3.00 ml of Eriochrome Cyanine R solution into a 100 ml volumetric flask. Dilute to about 85 ml with water and add from a pipet 10 ml of 70/30 HCl solution. Dilute to volume and mix well. In some cases variations in reagents may result in an abnormal standard curve (Low Absorbance readings for each standard). In this case dilute the reference solution with a known and accurately measured volume of water so that the absorbance readings are on scale. The absorbance of the 5 ml standard should be between 0.4 and 0.5 . The same reference solution used to prepare the standard curve must be used in analyzing samples.

16. From the absorbance, determine the milligrams of Fluoride by reference to the Standard Curve.

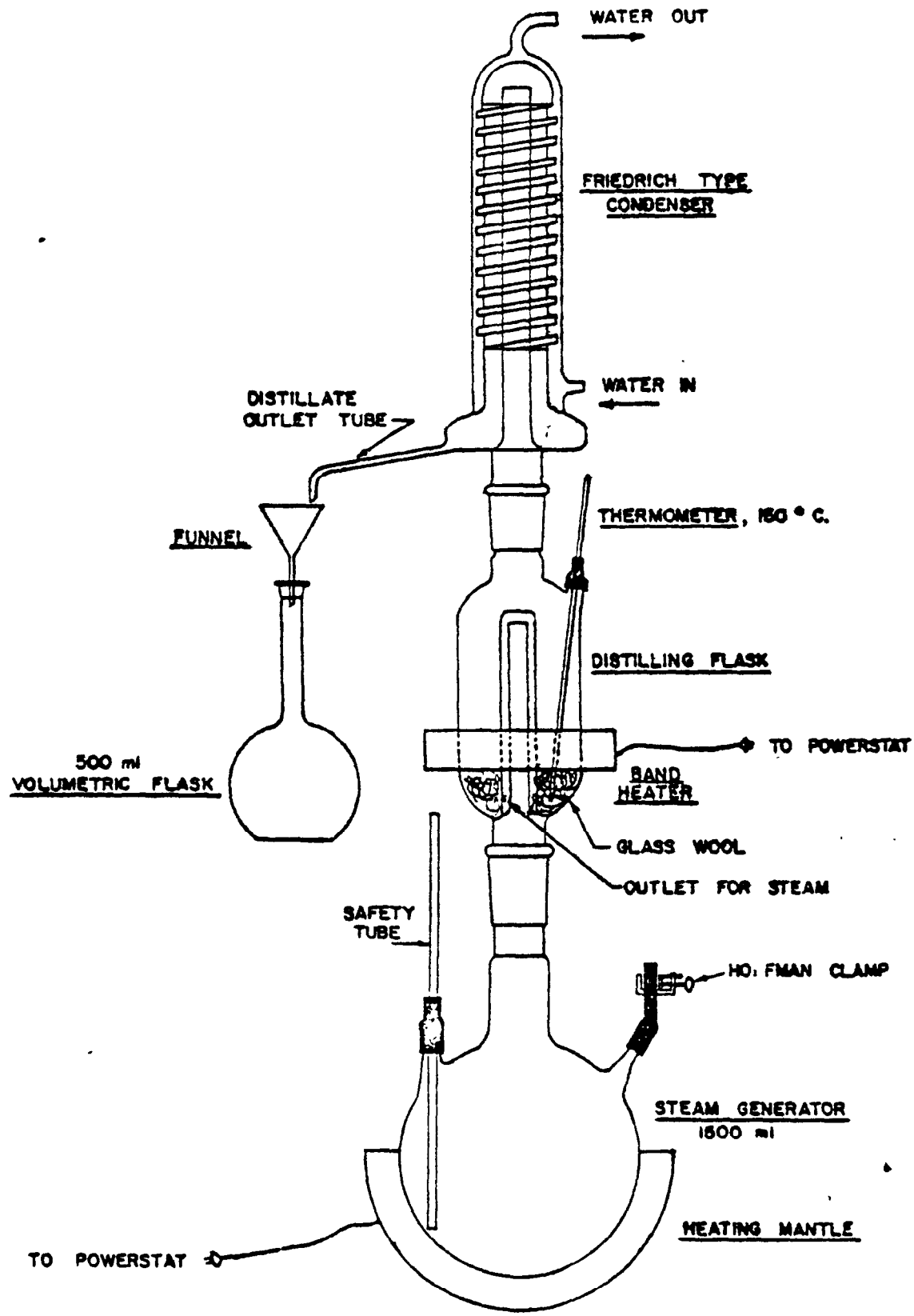
CALCULATION

1. $\frac{\text{(mg F from Curve) x 100}}{\text{Wt. of Sample x 1000 in Aliquot in grams}}$ = % Total Soluble Fluoride as F

step B.

FIGURE I

FLUORIDE STEAM DISTILLATION APPARATUS



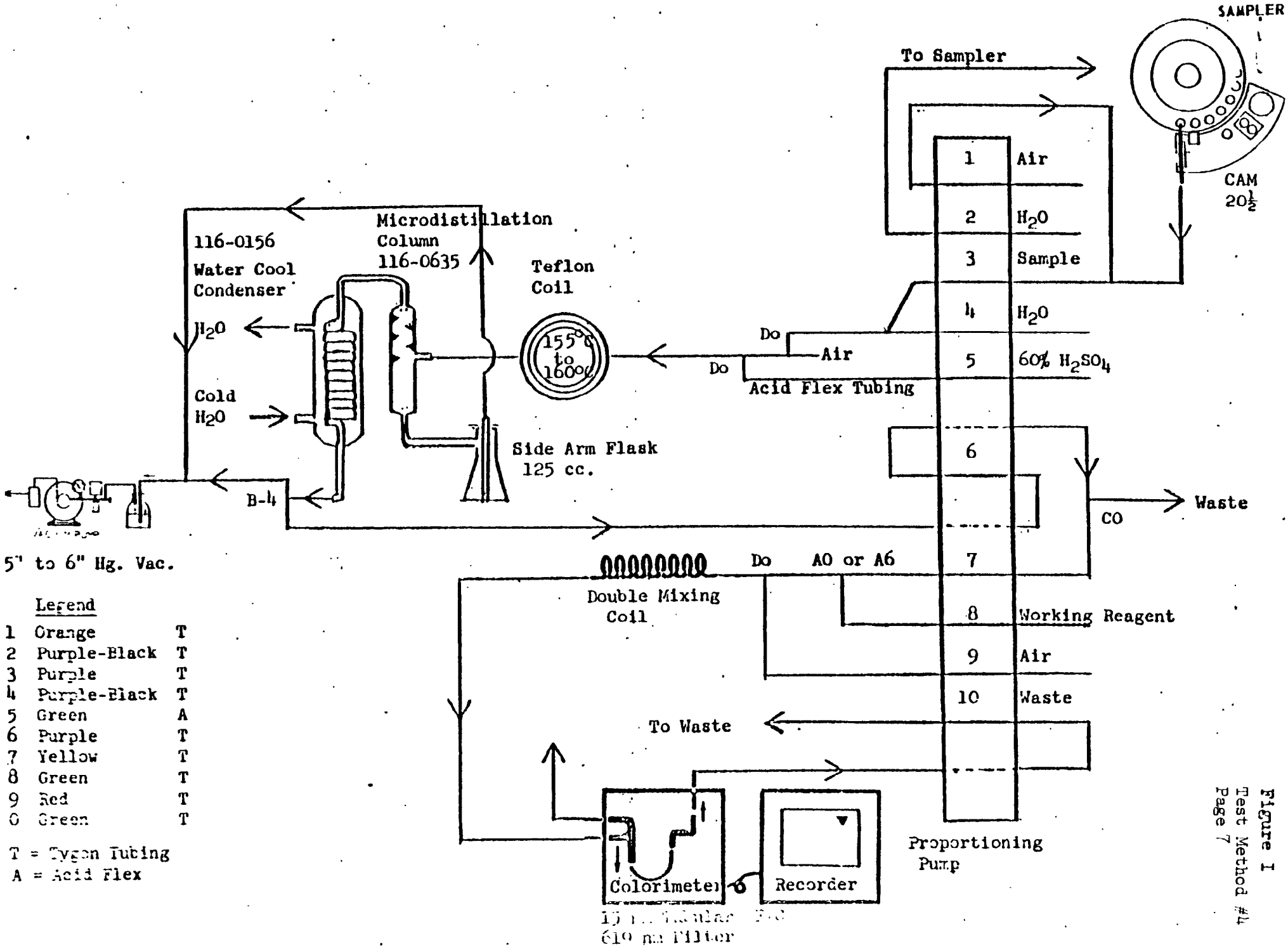


Figure 1
Test Method #4
Page 7

13

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #13

TITLE: DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #13

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

In addition to the previous methods, Total Soluble Available Fluorine can be calculated by adding the results obtained for soluble $PO_3F^{=}$ ion and soluble F^- ion.

14

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #14

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #14

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

The objective of this method is to measure the total soluble available fluorine in a dentifrice.

Reagents	Suggested Type or Source
n-Pentane 99%	Tridom Chemical
Toluene 99%	MCB Chromatoquality
HCl Conc.	Baker Analyzed reagent
Trimethylchlorosilane 99%	Tridom Chemical
NaF	Baker Analyzed reagent
Test tubes (screw-cap)	S.G.A.
Pipettes	S.G.A.

Apparatus	Suggested Type or Source
Gas Chromatograph Model 5710A with dual flame ionization detector	Hewlett-Packard

Column: 6 ft. 1/8" O.D. stainless steel packed with 25% DC-200 on Chromosorb NAW 80 - 100 mesh.

Temperature: Column = 90°C,
Detector = 250°C
Injection Port = 150°C

Carrier:

Nitrogen	= 30 ml/min	Matheson Gas Co.
Hydrogen	= 14 psig	Matheson Gas Co.
Air	= 24 psig	

Range: 64 x 10

Sample size: 0.6 ul of top layer

Notes on Method

This method is essentially the same as published by Cropper and Puttnams (J.S.C.C. 21,533,1970) for the determination of total fluoride in dental creams. It is based on an original publication of Bock and Semmler (Anal.Chem. 230,161,1967) and involves the following reaction:

$$R_3\text{-}SiCl + H_2O \longrightarrow R_3SiOH + HCl$$

$$R_3\text{-}SiOH + H^+ + F^- \longrightarrow R_3\text{-}SiF + H_2O$$

The alkyl-chlorosilane is converted by water into the corresponding silanol. The silanol reacts selectively with fluoride to form fluorosilane, which can be extracted from the acidified solution with an organic solvent. The extracted fluoro-compound is determined quantitatively by means of G.C. as described by Fresen et al. (Pharma.Weekblad 103,909,1968)

Procedure

Preparation of Solution (A)

Weigh 0.4422 gm of NaF. Transfer to a 1000 ml volumetric flask and fill to volume with deionized water (0.001 gm Fluorine/5 ml).

Preparation of Solution (B)

Weigh about 0.15 gm of n-pentane. Transfer to a 250 ml volumetric flask and fill up to volume with toluene (0.003 gm/5 ml).

Preparation of solution (C)

Pipette 5 ml of fluoride solution (A) into a screw-cap tube, add 8 ml of conc. HCl and 5 ml of deionized water. Cap and mix. Add 2 ml of trimethylchlorosilane, cap and mix. Let stand for 15 minutes. Pipette in 5 ml of n-pentane solution (B), mix and inject 0.6 ul of top layer.

Determination of Total Soluble Available Fluorine in Dentifrice

Weigh accurately 10.0 gm $\pm$ 1.0 gm of dentifrice into a centrifuge tube. Add approximately 20 - 30 ml of deionized water, slurry with a glass rod and centrifuge for 10 minutes.

Decant the supernatant liquid into a 100 ml volumetric flask. Repeat this extraction process an additional two times and bring the volume of the supernatant liquid up to 100 ml with deionized water.

Pipette 10 ml of the above solution into a screw-cap tube. Add 8 ml of conc. HCl and immerse in a boiling water bath for 1 minute and let stand for 10 minutes. Then cool under running water.

Add 1 ml trimethylchlorosilane, cap, mix and let stand for 15 minutes. Pipette 5 ml of n-pentane solution (B) into the test tube, shake and inject 0.6 ul of upper layer.

Calculation:

In the Standard Solution (C):

Factor =

$$\frac{\text{peak height of } F^- \text{ (TMFS)}}{\text{peak height of n-Pentane}} \times \frac{\text{gm of n-Pentane in 5 ml of Solution (B)}}{\text{gm of } F^- \text{ in 5 ml of Solution (A)}}$$

In the Dentifrice Sample:

gm of Fluorine =

$$\frac{\text{peak height of } F^- \text{ (TMFS)}}{\text{peak height of n-Pentane}} \times \frac{\text{gm of n-Pentane in 5 ml of Solution (B)}}{\text{Factor}}$$

$$\% \text{ Fluorine} = \frac{\text{gm of Fluorine} \times 10}{\text{weight of sample}} \times 100$$

PPM of Fluorine = % Fluorine X 10,000

15

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #15

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #15

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE IN A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

The objective of this method is to determine the total soluble available fluorine found in a sodium monofluorophosphate dentifrice.

Reagents	Suggested Type or Source
Sodium Hydroxide 4N	Baker Analyzed Reagent
Polyethylene Centrifuge Tubes	S.G.A.
Deionized Water	
Volumetric Flasks	S.G.A.
Glass Beaker	S.G.A.
HCl Conc.	Baker Analyzed Reagent
Pipettes	S.G.A.
Sodium Acetate	M.C.B.

Apparatus	Suggested Type or Source
Centrifuge Clinical	Damon/IEC Division
Ionalyzer Specific Ion Meter	Orion Research Inc.

Procedure:

Weigh accurately 8-12 gm $\pm$ 0.2 gm (w) of dentifrice into a glass beaker, slurry with 15-20 ml of 4 N sodium hydroxide and transfer carefully to a polyethylene centrifuge tube.

Centrifuge 5 minutes and decant into a polyethylene beaker. Slurry the residue again with 10-15 ml of deionized water. Centrifuge 5 minutes, decant and add to previous supernatant. Once more, slurry the residue with 10-15 ml of deionized water, centrifuge 5 minutes, decant and add to previous supernatant.

Acidify the combined supernatants with conc. HCl in small portions to a pH of 0.0 - 0.5. Add 2 ml excess of conc. HCl, cover and let stand 5-24 hours at room temperature. Transfer the solution quantitatively to a 100 ml volumetric flask and dilute to volume with deionized water.

Pipette 20 ml of the diluted sample solution into a 250 ml volumetric flask and again dilute to volume with deionized water.

Pipette 10 ml of this solution into a 100 ml volumetric flask, dilute to volume with sodium acetate solution (15% w/v) and mix thoroughly.

Standardize the specific ion meter by setting the center scale at 1 ppm fluorine and the upper end of the scale at 10 ppm fluorine using the two NaF solutions.

Using the meter, determine the reading in ppm (A) of the sample solution, immersing the specific electrode in about 40 ml of the solution contained in a polyethylene beaker and making due allowance for the electrode response time.

Calculation

$$\text{Total Soluble Available Fluorine (ppm)} = \frac{A \times 12,500}{W}$$

Where: A = ppm fluorine from meter reading
W = weight of dentifrice taken

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #16

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

Recommended for the following systems:

a. Sodium monofluorophosphate and silica abrasive.

TEST METHOD #16

DETERMINATION OF TOTAL SOLUBLE AVAILABLE FLUORINE

Principle

The toothpaste is extracted with water, and the fluoride content of the extract is determined.

Apparatus

Corning Digital 112 Research pH/mV Meter.
Orion Fluoride Specific-Ion-Sensitive Electrode 94-09A.
Orion Single-Junction Reference Electrode 90-01.
Magnetic stirring apparatus.
Volumetric flasks, 25-ml, 50-ml, 1000-ml sizes.
Pipets, 1-ml, 2-ml, 3-ml, 5-ml, 8-ml and 25-ml sizes.
Polyethylene beakers, 100-ml.
Petri dishes, 60 x 15 mm style.
Semi-log graph paper, 1 cycle.
Oven
Desiccator
Centrifuge, Servall Type SP, Ivan Sorvall, Inc.
Centrifuge tubes, round bottom, polypropylene, 50-ml capacity.
Centrifuge tubes closure to fit 50-ml centrifuge tubes.
Buret, 50-ml.

Reagents

Sodium hydroxide pellets, ACS Reagent Grade. Prepare 100 ml of 0.25N of a 3A alcoholic sodium hydroxide solution.
Sodium fluoride, ACS Reagent Grade. Prepare the solution shown below: Weigh accurately 2.2105 grams of reagent grade sodium fluoride. Dissolve and dilute to 1 litre in water (1000 ug/ml F^- standard). Pipet 10 ml of 1000 ug/ml fluoride standard into a 1000-ml volumetric flask, dilute to volume with water and mix thoroughly. (10 ug/ml F^- standard). Transfer the solution to a polyethylene bottle for storage.
Perchloric acid, 70% Reagent Grade.
Sodium citrate dihydrate, Reagent Grade.
Sodium chloride, Reagent Grade.
Glacial acetic acid, Reagent Grade.
Buffer solution: Dissolve 147 grams sodium citrate dihydrate and 58 grams sodium chloride in 1000 ml water. Add 60 ml glacial acetic acid. Mix by stirring. Adjust with sodium hydroxide (approx. 10% solution) to pH 5.5. Cool the solution then make up to 2 liters with water, and mix well. Store in a polyethylene bottle.
Calcium chloride - anhydrous, Reagent Grade.

Preparation of Standard Curve

Connect the lead of the fluoride electrode to the shielded input jack of the pH meter. Fill the reference electrode with the special filling solution supplied by Orion (No. 90-00-01). Do not use any other filling solution. Connect the lead of the reference electrode to the reference input jack of the pH meter. Turn the meter onto STANDBY function and place the electrodes in distilled water for about 15 minutes to allow them to equilibrate. Pipet 1 ml of 10 ug/ml fluoride standard and 25 ml buffer solution into a 50 ml volumetric flask (Note), dilute to volume with water, and mix well. Without using any additional water, transfer the contents of the flask into a clean 100 ml plastic beaker, draining the flask well. Carefully blot dry the electrodes with Kleenex tissues. Immerse the electrode tips in the buffered fluoride standard. Ensure that there are no air bubbles adhering to the electrode surface. Drop the stirring bar between them and stir at a moderate rate. Leave for 5-10 minutes until a constant potential is produced. Record the millivoltage. Remove the electrodes, rinse with distilled water and dry with Kleenex tissues. Determine the millivolt readings on the other standard solutions in a similar manner by replacing 1 ml of 10 ug/ml fluoride standard with 2 ml, 3 ml, 5 ml and 8 ml of 10 ug/ml fluoride standard respectively. Plot the calibration graph on semi-log paper with the millivolt reading on the linear abscissa and concentration on the logarithmic ordinate.

Preparation of Diffusion Dishes

Cover the inside of the Petri dish cover with a known amount of sodium hydroxide by placing 0.3 ml of 0.25N ethanolic sodium hydroxide in the lid, covering the whole surface by a gentle rotating movement. Evaporate the alcohol in a desiccator containing calcium chloride to leave a perfectly regular adherent layer. If the layer is not regular do not use the dish, but prepare a fresh one. Dishes may be prepared in advance, but must be stored in a desiccator containing anhydrous calcium chloride.

Procedure

Weigh about 2 grams of paste to an accuracy of ± 1 mg into a centrifuge tube. Add from the 50 ml buret small increments of distilled water, thoroughly mixing with a small glass rod after each addition until a smooth slurry is obtained. Continue the addition of water until the total volume added is 10 times the weight of the paste taken. Cap the tube and centrifuge at 4000-5000 rmp for 15 minutes. Immediately decant the supernatant liquid into a 50 ml polyethylene bottle. Pipet 5 ml of this solution into a 25 ml volumetric flask and dilute to volume with water. Pipet 2 ml of sample solution into the lower part of the Petri dish. Add 4 ml of 70% perchloric acid and cover immediately with the prepared lid. Heat in an oven at 60°C overnight. Remove the dish from the oven and immediately remove the cover. Wash the inside of the cover about five times with a few ml of water, catching the wash in a 50-ml volumetric flask (Note) containing 25 ml of buffer. Make up to volume with water. Read the potential in millivolts, following the procedure used for the standard solutions.

Note: A glass volumetric flask may be used if the solution is transferred to a plastic container after diluting to volume.

Calculations

Determine the fluoride concentration in the sample solution by referring to the standard curve.

$$\% \text{ Available Fluoride (1:3 dilution)} = \frac{J \times D \times 25 \times 100}{W \times 2 \times 2 \times 10^6}$$

$$= \frac{J \times D}{W \times 1600}$$

J = ug of fluoride read from the standard curve.

W = Weight of sample in grams.

D = ml of water used to prepare slurry.

TEST METHOD #17

Title: Determination of Soluble Tin(II) in Stannous Fluoride - Calcium Pyrophosphate Toothpaste by the Iodate Titration Method

Recommended for the Following System:

a. Stannous Fluoride - calcium pyrophosphate

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #32

TITLE: DETERMINATION OF pH

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #32

DETERMINATION OF pH

Using a pH meter which has been standardized with pH 4.0 and 7.0 buffer solutions, measure the pH of a portion of the dental cream.

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #31

DETERMINATION OF THE pH OF A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK

METHOD #31

DETERMINATION OF THE pH OF A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

Objective:

To measure the pH of a sodium monofluorophosphate chalk dentifrice.

Reagents	Suggested Type or Source
Buffer Solution pH: 7 & 10	Harleco
Deionized Water	
Polyethylene Beaker (100 ml)	S.G.A.

Apparatus

Corning Model 10 pH Meter	Corning - Distributed by Scientific Glass Apparatus Co.

Procedure

1) Weigh 5.0 gm ± 0.2 gm of dentifrice into a 100 ml beaker.

2) Slurry with 20 ml ± 0.1 ml of deionized water.

3) Standardize the meter with appropriate buffer solution to cover pH range for 7 to 10.

4) Check the pH of the dentifrice slurry with the pH meter.

X

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #34

DETERMINATION OF ENAMEL SOLUBILITY REDUCTION

Recommended for the following systems:

a. Stannous fluoride and silica abrasive.
b. Stannous fluoride and calcium pyrophosphate abrasive.
c. Sodium monofluorophosphate and silica abrasive.
d. Sodium monofluorophosphate and calcium carbonate abrasive.

TEST METHOD #34

DETERMINATION OF ENAMEL SOLUBILITY REDUCTION

The reduction in enamel solubility method is adapted from the method of Hefferren.[1] Modifications have been made in order to obtain a more rapid and convenient procedure.

Outline of Method

Sound, excised teeth are mounted in acrylic donuts and briefly exposed to lactate buffer to remove the outer layers of enamel (which may be rich in fluoride). The teeth are then subjected to an acid dissolution with lactate buffer for a given period of time, and the amount of enamel dissolved from the tooth is measured. The stannous fluoride product to be tested is then applied to the teeth. Subsequently a second measurement of the amount of enamel dissolved from the tooth is made. A comparison of the amount of enamel etched off the tooth prior to stannous fluoride treatment with that removed after stannous fluoride treatment gives the percent reduction in enamel solubility.

Methods and Materials

The following solutions and equipment are required:

a) 1.0M lactic acid-solution lactate buffer solution, pH = 4.5 (refer to method of Hefferren[1] for preparation of buffer).

b) Reduced molybdate solution - see Appendix A for preparation.

c) Phosphorous standards - 2, 5, 10 and 20 ug P/ml - see Appendix A.

d) constant temperature (37°C) water bath

e) water driven immersible magnetic stirrers (G. Frederick Smith Chemical Co., Ace Cat No. 22-2435/01).

f) one 50 ml, one 5.0 ml, and one 10.0 ml capacity Repipet (Labindustries)

g) 2.0 ml volumetric "class A" pipets

h) 150 x 17 mm test tubes

i) Army medical water bath (Ace Scientific)

A. Preparation of teeth

Teeth are prepared as follows:

1. For each product to be tested, twelve noncarious excised teeth (usually molars) are employed. Remove debris and residual tissue from the crown portion of the tooth with a dental scaler. Pumice each crown (flower of pumice) for 30 seconds to assure complete removal of extraneous matter.

2. Cut the teeth (cutting disc) approximately 2-3 mm below the dento-enamel junction.

B. Construction of tooth mount

3. Add into a small paper cup dental acrylic powder and acrylic liquid in proportions that will give a slightly thickened mixture (small bubbles will appear in the acrylic). Pour the acrylic mixture into a plastic disposable dish having an inner diameter of 40-45 mm. The depth of the acrylic in the dish should be about 5 mm. When the acrylic begins to set, place four enamel crowns, occlusal portion up, into the acrylic in a circular manner. The dento-enamel junction should be completely covered by the acrylic. When the acrylic has set, remove the "donut" from the dish. Place the donut into an aluminum weighing dish (Ace #12-1570/01) and, with an eyedropper, cover the acrylic with Kerr blue inlay wax so that top, sides and bottom are covered. Allow wax to harden and remove the donut from the aluminum dish. Pipet 50.0 ml of water into a 5 oz. plastic cup, 62 mm top diameter by 42 mm base diameter. Trim the sides of the wax-covered acrylic donut until the donut just comes in contact, enamel crown side down, with the surface of the water and the crowns are completely immersed. Bore a hole in the center of the donut just large enough to accommodate a thermometer.

C. Conditioning of enamel crowns

4. Prior to the start of an RES run, condition the enamel crowns by inserting the acrylic donut crown side down into a 5 oz. plastic cup containing 50.0 ml of 0.1M lactate buffer (prepared by diluting the stock 1.0M lactate buffer by a factor of 10). Expose the crowns to this buffer for 45 minutes, discard the buffer and repeat the 45-minute exposure using fresh buffer. Subsequent to this 1.5 hr. conditioning period, brush the teeth lightly under running distilled water. Store in deionized water until ready for pre-treatment etch.

D. Pre-treatment etch

5. Dispense (Repipet) 50.0 ml of 0.1M lactate buffer into clean 5 oz. plastic cups. Place a magnetic stirrer into each cup and place each cup onto a water-driven magnetic stirrer (GFS Chemical Co) submerged in a constant temperature (37°C) water bath. Dry the tooth mounts using a stream of nitrogen, and place the mounts tooth side down into the buffer-containing cups when the temperature of the buffer reaches 37°C. Expose the teeth to the buffer for exactly 15 minutes. At the end of this period, remove the tooth mounts from solution and rinse under running distilled water. Store in deionized water until exposure to dentifrice slurry (Section E). Take a 2.0 ml aliquot (class A volumetric pipet) of the buffer solution containing the dissolved enamel and pipet into a 150 x 17 mm test tube. Seal the top of the tube with Parafilm and save for phosphorous analysis (Section H).

E. Exposure to Dentifrice Slurry (or fluoride solution)

Prepare 25% (w/w/) slurries of the dentifrices to be tested. Dispense 50.0 ml of the slurries into 5 oz. plastic cups, place in the water bath and allow the slurry temperature to reach 37°C (with stirring). Dry the tooth mounts using a stream of nitrogen and place crown side down into the slurry for exactly five minutes. At the end of this time, remove the tooth mounts and brush lightly under running distilled water. Store in deionized water until post-treatment etch.

F. Post-treatment etch

Repeat exactly the procedure followed for the pre-treatment etch. Save 2.0 ml aliquot for phosphorous analysis.

G. Analysis of phosphorous (Lucena-Conde Method)

Phosphorous Standards (see Appendix A): Pipet 2.0ml of the phosphorous (KH_2PO_4 Fisher Certified Reagent P-285) standards solutions into 150 x 17 mm test tubes. Concentrations of 2.5, 5.0, 10.0 and 20.0 ug P/ml are recommended for the range of phosphorous levels encountered in the assay.

Color development: To the 2.0 ml aliquots of sample (or standard), add 1.5 ml of reduced molybdate reagent and 10.0 ml of deionized water. Shake vigorously using a vortex mixer (Ace Scientific Cat No. 22-2430) and place test tubes in boiling water for 45 minutes. Remove tubes and allow to cool to room temperature. Read absorbance at 820nm (Beckman DBGT Spectrophotometer) against a blank sample (2.0 ml aliquot of lactate buffer, 1.5 ml molybdate reagent, 10.0 ml deionized water, heat in boiling water for 45 minutes).

H. Determination of RES from absorbance data

Plot absorbance vs. phosphorous concentration for the phosphorous standards employed to assure that a linear relationship exists between the two parameters.

Determine RES directly from the absorbance values obtained for each sample:

$$\% \text{ RES} = \frac{A-B}{A} \times 100$$

where A = inital (pre-treatment) absorbance value
B = final (post-treatment) absorbance value

REFERENCES

1. Hefferren, John J., "Interfaces of Laboratory and Clinical Assessment of Therapeutic Dentifrices," J. Soc Cosmet Chem, 24:815-828 (1973).

APPENDIX A

PREPARATION OF STANDARDS AND SOLUTIONS REQUIRED FOR PHOSPHOROUS ANALYSIS USING THE LUCENA-CONDE METHOD

A. Preparation of Phosphorous Standards

Dry a sample of KH_2PO_4 analytical reagent (Fisher Certified Reagent P-285) for 3 hrs. at 105°C. Dissolve 2.194 grams in deionized water and dilute to 500 ml in a volumetric flask. The phosphorous concentration of the stock standard solution is 1.0 mg P/ml.

Working standards - Prepare phosphorous standards in the range of 0 to 10 ug P/ml:

1.0 ug P/ml: 0.10 ml of 1.0 mg P/ml, stock solution, dilute to 100 ml in volumetric flask.

2.5ug P/ml: 0.25 ml of 1.0 mg P/ml stock solution, dilute to 100 ml

5.0ug P/ml: 0.50 ml of 1.0 mg P/ml stock solution, dilute to 100 ml

10.0ug P/ml: 1.00 ml of 1.0 mg P/ml stock solution, dilute to 100 ml

Take 2.0 ml aliquots of these standards and color develop in the same manner used for the aliquots of lactate etch solution.

B. Preparation of Reduced Molybdate Solution (for color development of phosphorous samples)

1. Transfer 40.75 g of ammonium molybdate (ACS reagent crystal) to a 500 ml Erlenmeyer flask and dissolve in 300 ml of deionized water.

2. Transfer to a 500 ml glass stoppered Erlenmeyer 125ml of the molybdate made in step 1, add 125 ml of 6N HCl to give a total volume of 250 ml.

3. Mix and add 50 ml of mercury.

4. Shake well and filter the mixture to get a reddish brown reduced molybdate solution.

5. Carefully transfer to a 1-l volumetric flask 150 ml of the molybdate solution made in step 1. Add 250 ml of concentrated HCl and mix. Add 250 ml of concentrated H_2SO_4 and mix. To this mixture add 200 ml of the reduced molybdate solution from step 4. Fill the flask to the liter mark with deionized water. The final solution is green and is stable for six months.

References:

1. Lucena-Conde, F, and Prat, L., "A reagent for the determination of P, As, and Ge," Anal Chim Acta 16:473 (1957).

2. Marin, J. B., and Doty, D. M., "Determination of Inorganic Phosphate - modification of iso - BuOH procedures," Anal Chem 21:965 (1949).

TEST METHOD #33

Title: Determination of Enamel Solubility Reduction (ESR)

Recommended for the Following Systems:

a. Stannous Fluoride - calcium pyrophosphate

b. Stannous Fluoride - insoluble sodium metaphosphate

c. Sodium Fluoride - high-beta-phase calcium pyrophosphate

TEST METHOD #33

Determination of Enamel Solubility Reduction (ESR)

Teeth

The test is run on sound human teeth, usually molars, cuspids, and bicuspids. Upper and lower incisors are not normally used. Sound teeth having no fillings or decay are selected and scraped with a scalpel to remove the remaining flesh and heavier calcified deposits. The lighter deposits and stains are taken off by polishing with a handpiece and pumice abrasive. The roots of the teeth are then cut off about one-fourth inch below the lowest point of the enamel. Any part of a tooth which appears damaged is covered with blue inlay casting wax, and a hot probe is touched to the wax at this spot to adhere the wax to the enamel. The teeth are sorted into groups of six, each group containing at least one of each type of tooth.

Mounting

The teeth are mounted in 180-ml tall-form beakers. Three indentations are made in each beaker, one-fourth inch from the bottom. Blue inlay casting wax is melted and kept on a hot plate at about 340°F. A small amount of wax is placed in the bottom of the mounting beaker, swirled around, and allowed to harden, so that the inside of the beaker is covered with wax up to the indentations. A layer of dry-phase F-88* (a two-phase epoxy cement) about one-eighth inch in depth is placed evenly over the blue wax. F-88 liquid is dropped on the powder until the powder is completely dampened. This adhesive mixture is allowed to stand until it begins to thicken. A matched set of six teeth is dried, and the teeth are evenly spaced in the adhesive with the enamel ends up. After the adhesive mixture has partially dried and the mounted teeth are solidly in place, more inlay wax is added to the beaker to cover the cement and the exposed roots of the teeth. When the wax has cooled, a hot probe is used to draw wax up over any dentin which remains exposed. The tooth set is covered with water immediately after completion of these operations.

Lactate Buffer

The lactate buffer used in this test is prepared by diluting 2 moles (by weight) of lactic acid with approximately 400 ml of water. To this is added a solution consisting of 84 g of NaOH dissolved in about 600 ml of water. The solution is diluted to 2000 ml and stirred.

A 1 molar lactic acid solution is prepared by diluting 2 moles (by weight) of lactic acid to 2000 ml with water. The solution of lactic acid and sodium hydroxide is placed in a large beaker, and pH electrodes are placed in the liquid. The pH of this solution should be above 10.5. The 1 molar lactic acid solution is added until the pH of the buffer is 4.5. The final buffer is 1.0 M in lactate. To obtain a 0.1 M working concentration of the buffer, the 1M buffer is diluted by a factor of 10 with water.

Deprotection

Test runs are made on sets of four mounts, that is, four beakers of six teeth each. Before every use, any residual anti-solubility protection afforded by the previous treatment must be removed. Deprotecting is accomplished by etching in the above prepared lactate buffer solution for 2 hours. About 100 ml of lactate buffer is sitrred in the toothset beaker with a constant speed (1725 rpm) motor. The

*American Consolidated Mfg. Co., Philadelphia, Pa.

buffer must be renewed for each one-hour deprotection period. If more than 8 hours has elapsed since the last deprotection, an additional 15-minute deprotection period is required.

Test

The test is run with the tooth mounts immersed about half their beaker heights in a constant-temperature water bath set at 37°C. The deprotected tooth sets are held in clamps which maintain them at this height in the bath. Constant-speed motors (1725 rpm) are lowered so that their stirring propellers are about one-half inch above the teeth in the beakers. The height of the propeller above the teeth must be constant throughout the test. A 40-ml portion of lactate buffer is added to the first set, and the clock and stirrer are started. At 30-second intervals the other three sets are started in the same manner. After 15 minutes of exposure to the acidic solution, the first set is stopped, and the lactate buffer is poured into a 2-ounce sample bottle. The other three sets are treated likewise at 30-second intervals. The tooth mounts are rinsed three times in distilled water and replaced in the water bath for the treatment step.

A 15-gram portion of dentifrice is weighed out, 45 ml of water is added, and these are slurried together. About 50 ml of this slurry is centrifuged for 15 minutes.

A 40-ml portion of the supernatant from the dentifrice slurry is added to the first tooth set, and the stirrer and the clock are started. At one-minute intervals, the other three sets are started in the same manner. At the end of five minutes of treatment, the first set is stopped, the treatment solution discarded, the stirring propeller rinsed, and the tooth set rinsed well in running distilled water. The other three sets are removed at one-minute intervals and treated likewise.

A second acid exposure is performed by the same method as the first with the lactate buffer solutions being retained. The pre-treatment and post-treatment acidic solutions are analyzed for phosphorus using a modified Martin-Doty method on the AutoAnalyzer.**

Calculation of E.S.R.

The percent of enamel solubility reduction is computed as the difference between the amount (ppm) phosphorus in the first and second acidic solutions divided by the amount (ppm) phosphorus in the first solution and multiplied by 100.

** Technicon Instruments Corporation, Chauncey, New York

STANDARDS FOR FLUORINE DENTIFRICES

TEST METHOD #35

DETERMINATION OF THE ENAMEL SOLUBILITY REDUCTION BY A SODIUM MONOFLUOROPHOSPHATE CHALK DENTIFRICE

RECOMMENDED FOR THE FOLLOWING SYSTEM

SODIUM MONOFLUOROPHOSPHATE-CHALK